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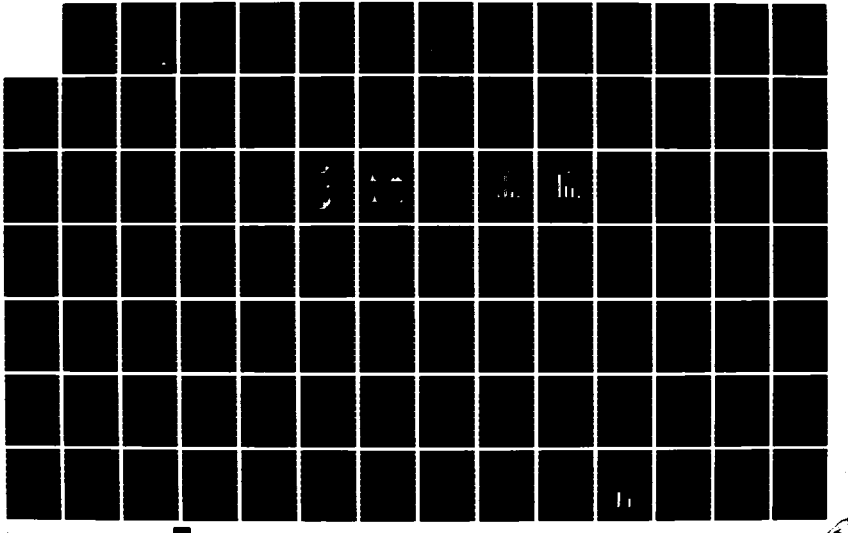

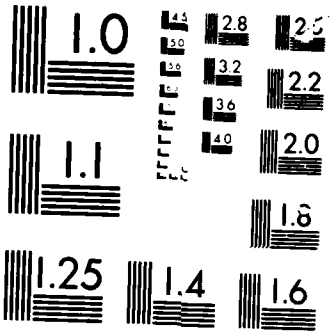

USAFSAM-TR-85-105

EFFECTS OF LONG-TERM RADIOFREQUENCY RADIATION ON IMMUNOLOGICAL COMPETENCE AND METABOLISM

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NOTICES

This final report was submitted by the Bioelectromagnetics Research Laboratory, Center for Bioengineering, School of Medicine, University of Washington, Seattle, Washington, under contract F33615-83-C-0620, job order 7757-01-1A, with the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks AFB, Texas. Dr. Jerome H. Krupp (USAFSAM/RZP) was the Laboratory Project Scientist-in-Charge.

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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Two groups of 20 rats were exposed 21 h/day for 6 and 12 mo, respectively, to 10-μs-pulsed 2450-MHz microwaves at 600 pps and 8-Hz modulation, at an average power density of .48 mW/cm^2 (SAR 0.15-0.4 W/kg) in circularly polarized waveguides. Equal numbers of rats were sham exposed. Effects seen in the previous study (elevated corticosterone level after 6 wk exposure; increased B and T cells in rats at 13 mo exposure) were not replicated in this study. Quantitative immunological tests involving 70 parameters showed six different effects. Among them, increased hematopoietic progenitor cells in the marrow was the only consistent effect in both 6- and 12-mo exposed groups. In the second part of the study three groups of rats were exposed 21 h/day to 2450-MHz CW microwaves at an average SAR of 0, 2.5, 5, and 7.5 W/kg (10 rats each) under three environmental temperatures (17.8, 22.2, and 26.7°C) over three 6-wk periods. Metabolism--including body mass, food/water intake, oxygen consumption, and carbon dioxide production--was measured, and health profile and immunological response were monitored. Results show that the effects are highly dependent on exposure level and environmental temperature.

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SUMMARY

This report presents the results of two studies on effects of long-term radiofrequency radiation of rats. The first was an attempt to replicate the finding of enhanced immunological response after exposure to pulsed microwaves. The second study was to generate, in a parametric fashion, a dose-response demonstration of the effects on metabolism, health profile, and immunological response.

Two groups of 20 male Sprague Dawley rats were exposed to 10-μs-pulsed 2450-MHz microwaves at 800 pps and 8-Hz modulation for 21 h/day for 6 or 12 mo, at an average power density of 0.48 mW/cm^2 (SAR 0.15 to 0.4 W/kg) in circularly polarized waveguides. When compared to data from an equal number of sham-exposed rats, the following results were obtained:

1. No effect on body mass or food and water consumption
2. Effects on immune system from the 6-mo exposure:
   a. Increased number of marrow hematopoietic progenitor cells
   b. Increased proliferative responses to purified pokeweek derivative by splenic B cells
   c. Decreased total B lymphocytes in marrow
   d. Decreased proportion of small cells in marrow
3. Effects on immune system from the 12-mo exposure:
   a. Increased number of hematopoietic precursors
   b. Reduction in cell surface expression of Thy 1.1 surface antigen on thermocytes
   c. Reduction in the mean cell-surface density of s-Ig on small lymphocytes in spleen
4. No effect on hematology, serum chemistry, thyroxine, or protein electrophoresis
5. Negative effect on corticosterone level
6. No significant difference (pathological examination) between exposed and control animals except an increased peribronchiolar lymphoreticular proliferation after 12-mo exposure
7. Tumor incidence too low to allow meaningful statistical comparisons

The second part of the project involved exposing three groups of rats--at three environmental temperatures (17.8, 22.2, and 26.7°C respectively)--to 2450-MHz CW microwaves for 21 h/day in circular waveguides at average power densities of 0, 5, 10, and 15 mW/cm^2 (10 rats from each group), corresponding to SARs of 0, 2.5, 5, and 7.5 W/kg, over three 6-wk periods. Metabolism--including body mass, food and water intake, oxygen consumption, and carbon dioxide production--was measured. Health profile and immunological response were also monitored. Results are summarized below:

1. Mortality occurred at high exposure levels, especially at high temperature.
2. Colonic temperature indicated that a 1°C chronic body-temperature rise was the survival threshold.
3. Temperature rise in the metabolism cage was a complex function of power level, temperature, and animal response.
4. Both power level and temperature affected growth.
5. Food consumption decreased as power density increased, at all temperatures.
6. Effect on water consumption was not consistent.
7. Effects on serum chemistry were mostly due to temperature change.
8. Radiation consistently affected BUN, ionized calcium, and alkaline phosphatase.
9. Hematology and protein electrophoresis showed no effect.
10. At 17.8°C, radiation caused corticosterone level to rise; at 22.2°C and 10 or 15 mW/cm^2, the corticosterone level decreased, probably due to adrenal exhaustion.
11. Both oxygen consumption and carbon dioxide production decreased during all exposures. The decrease was also a function of temperature.
12. Effects on immune system were dramatic. They included
    a. pronounced radiation effect on cellularity of the spleen,
    b. biphasic response on the cellularity of the thymus gland,
    c. effects on hematopoietic progenitor cells, and
    d. increased B cells in the thymus.

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TABLE OF CONTENTS

List of Illustrations

List of Tables

EFFECTS OF LONG-TERM RADIOFREQUENCY RADIATION ON IMMUNOLOGICAL COMPETENCE AND METABOLISM

INTRODUCTION

During 1978-1983 the Bioelectromagnetics Research Laboratory at the University of Washington conducted the largest microwave-bioeffects evaluation study ever undertaken. The goal of that project was to investigate purported adverse health effects of long-term exposure to pulsed microwave radiation. The major emphasis was on exposing a large population of experimental animals to microwave radiation throughout their lifetimes and monitoring them for cumulative effects on general health and ultimately on longevity (Guy et al., 1983a). In this follow-up study, we have focused on the effects on immunological competence and metabolism.

A brief summary of the previous project will provide a background for the present study. A unique exposure facility was prepared that allowed 200 rats to be maintained under specific-pathogen-free (SPF) conditions while housed in individual circularly polarized waveguides. These waveguides had been developed in this laboratory as a relatively inexpensive exposure apparatus that provided highly quantifiable microwave exposure conditions (Guy and Chou, 1976; Guy et al., 1979).

The exposure facility consisted of two rooms, each containing 50 waveguides for active exposures and 50 for sham exposures. Each room contained two 2450-MHz pulsed microwave generators, each able to deliver a maximum of 10-W average power at 800 pps with a 10-μs pulse width. This carrier was square-wave modulated at an 8-Hz rate. The power distribution system delivered 0.144 W to each exposure waveguide, for an average power density of .48 mW/cm^2. Whole-body calorimetry, thermographic analysis, and power meter analysis indicated that these exposure conditions resulted in average specific absorption rates (SARs) ranging from approximately 0.4 W/kg for a 200-g rat to 0.15 W/kg for an 300-g rat (Chou et al., 1984).

At 3 wk of age, 200 male rats were obtained from a commercial barrier-reared colony and randomly assigned to exposed and sham-exposed treatment conditions. Exposure began at 8 wk of age and continued for 25 mo. Throughout this period all surviving animals were bled at regular intervals and blood samples were analyzed for a panel of serum chemistries, hematological values, protein electrophoretic patterns, and thyroxine and plasma corticosterone levels. Body mass and food and water consumption were measured daily, and oxygen consumption and carbon dioxide production were periodically measured in a subpopulation of the exposed and sham-exposed groups. Activity in an open-field apparatus was assessed at regular intervals throughout the study. After 13 mo, 10 rats from each treatment condition were killed for immunological competence testing, whole-body analysis, and gross and histopathological examinations. The surviving 23 rats (11, sham-exposed group; 12, exposed group) were killed at the end of 25 mo for similar analyses.

The design and results of that study have been published in a series of nine technical reports covering major subtopics (Guy et al., 1983a, 1983b, 1985; Chou et al., 1983b; Johnson et al., 1983, 1984; Kunz et al., 1983, 1984, 1985). Among the 155 parameters measured during the study, most showed no statistical difference. The few endpoints that showed statistical differences were as follow:

1) Serum corticosterone values in exposed animals were elevated at the time of the first sampling session (6 wk exposure).
2) When compared with the sham-exposed group at 13 mo, the exposed animals had a significant increase in both splenic B and T cells; and mitogen-stimulation studies revealed a significantly increased response to concanavalin A (Con A) and a decreased response to pokeweed mitogen (PWM) and purified protein derivative (PPD).
3) The exposed group had statistically more total primary malignant tumors.

In this follow-up study, the first two effects were repeated, but not the third which requires an experiment of a considerably different design.

The technical reports referenced in the last paragraph provide details of the exposure facility, dosimetry, and animal procedures.

OBJECTIVES

The major objective of the current study was to replicate the finding of enhanced immunological response after exposure to pulsed microwave radiation and to determine the temporal variation of the observed effects after 6- and 12-mo exposures. All research was conducted under experimental conditions identical to those in the original experiment, with an enlarged sample size and refined assay techniques.

A second objective was to generate, in a parametric fashion, a dose-response demonstration of effect for those endpoints where no effects were observed at the low power density used in the original experiment. Particular emphasis was placed on measures of metabolism.

SCOPE OF WORK

We replicated the exposure conditions under which enhanced immunological responses had been observed. Two groups of 20 animals each were exposed for 6 and 12 mo respectively. An equal number of sham-exposed animals served as controls. For best use of the existing exposure facility, we started the 6- and 12-mo exposure groups at the same time in one exposure room. To replicate the experimental procedures used in the original study, we conducted regular bleedings at 6-wk intervals during the exposure periods; however, these blood samples were analyzed only at the final bleeding immediately prior to sacrifice of animals for removal of spleen, thymus, and bone marrow for immunological testing. As in the original study, we determined B- and T-cell enumeration and mitogen-stimulation responses of the splenic cells. We also measured the response of thymic lymphocytes and marrow stem cells. Gross pathological and histopathological evaluations of each animal were made following sacrifice.

To determine a dose-response relationship for demonstrable microwave effects, we exposed nine groups of 10 rats each to one of three power densities at one of three ambient temperatures for up to 6 wk.

Ten sham-exposed animals were used as control subjects for each ambient temperature. We collected measurements of metabolism including body weight, food and water consumption, and respiratory gas exchange. Serum chemistry, hematology, protein electrophoresis, thyroxine, and corticosterone were measured at death. Following sacrifice of the animals we made gross pathological and histopathological examinations and removed spleen, thymus, and bone marrow tissue for immunological competence testing.

IMMUNOLOGY

The biological endpoints evaluated in this phase of the project are concerned directly with the immune competence evaluation, including serum chemistry, hematology, protein electrophoresis, thyroxine, corticosterone, and gross pathological and histopathological examinations. These endpoints are the same as those in the original project. As part of the replication, we measured body mass and food and water consumption daily.

Body Mass and Consumption of Food and Water

The animal population, housing facilities, and maintenance procedures are described in detail in Volume 1 of the original project (Guy et al., 1983a). At 21 days of age, 100 male SPF rats (Sprague-Dawley) were obtained from a commercial supplier (Camm (SD) BR, Camm Research Labs, Wayne, NJ) and housed individually throughout the study in a barrier facility. For 21 h/day these animals were in Plexiglas cages placed inside wiremesh waveguides; 50 under microwave-exposure and 50 under sham-exposure conditions. Ten rats in each group served as spare animals. Each morning all animals were removed from these cages at staggered times over a 2-h period, weighed, and placed in standard filter-bonneted Plexiglas cages while their waveguide cages were being cleaned. The facility was maintained at 21 $\pm$ 1°C and 55 $\pm$ 10% relative humidity. Fluorescent room lighting was maintained on a 12/12 light/dark cycle (lights on 0700-1900).

During the 21 h/day that the animals were housed in the waveguide, they had ad libitum access to autoclaved pelleted chow (Certified Autoclavable Rodent Chow #5014, Ralston Purina Co., St. Louis, MO) and distilled water.

Figures 1 and 2 show the mean body mass of the 6- and 12-mo exposures. Analysis of variance with repeated measures did not reveal any significant difference ($p > 0.05$). The same results are observed on food and water consumption, as shown in Figures 3-6. No effect was found.

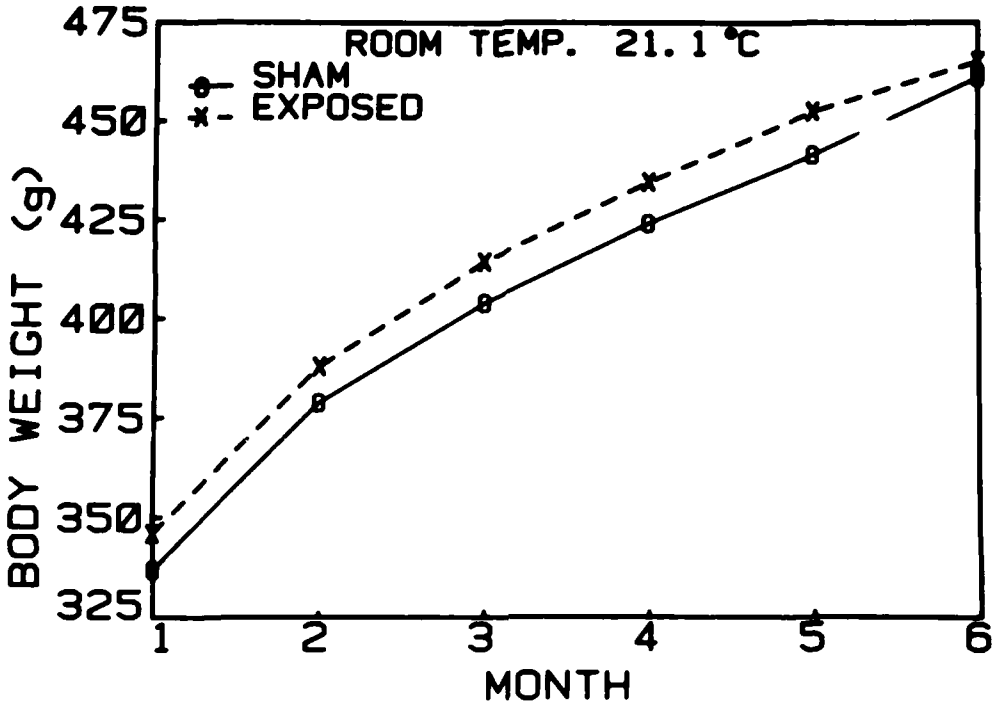

Figure 1. Mean body mass of the 6-mo exposure.

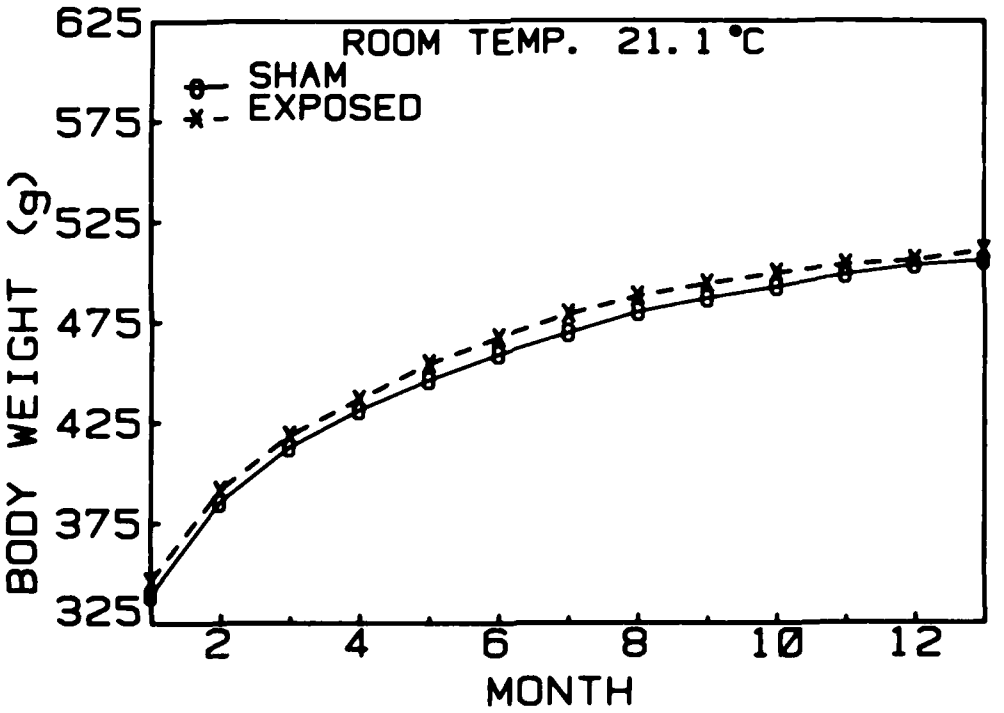

Figure 2. Mean body mass of the 12-mo exposure.

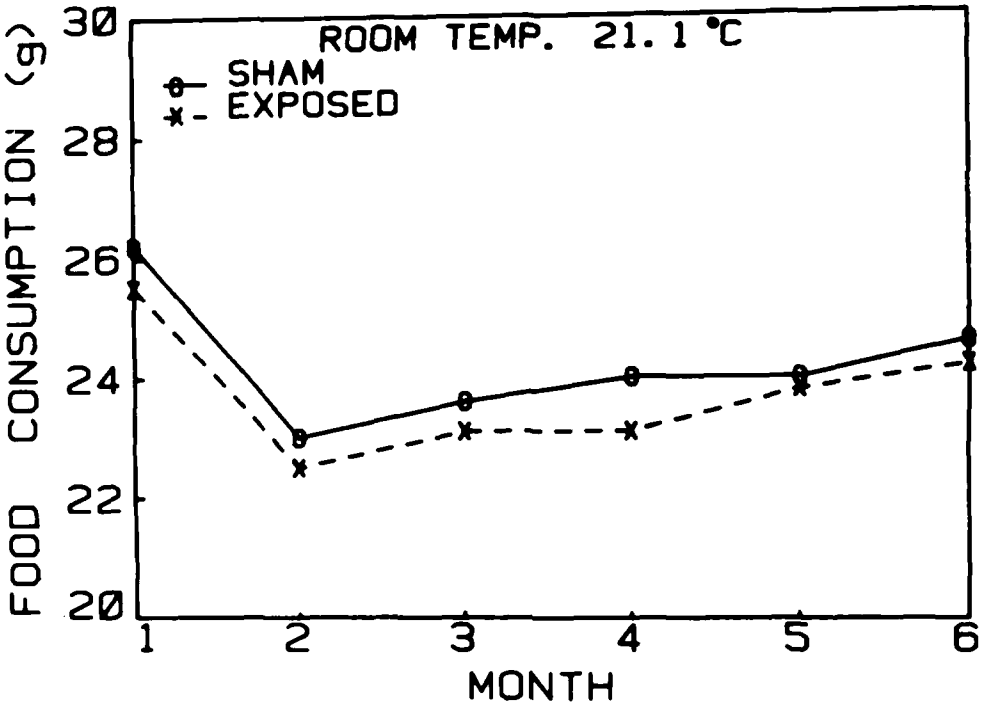

Figure 3. Mean food consumption of the 6-mo exposure.

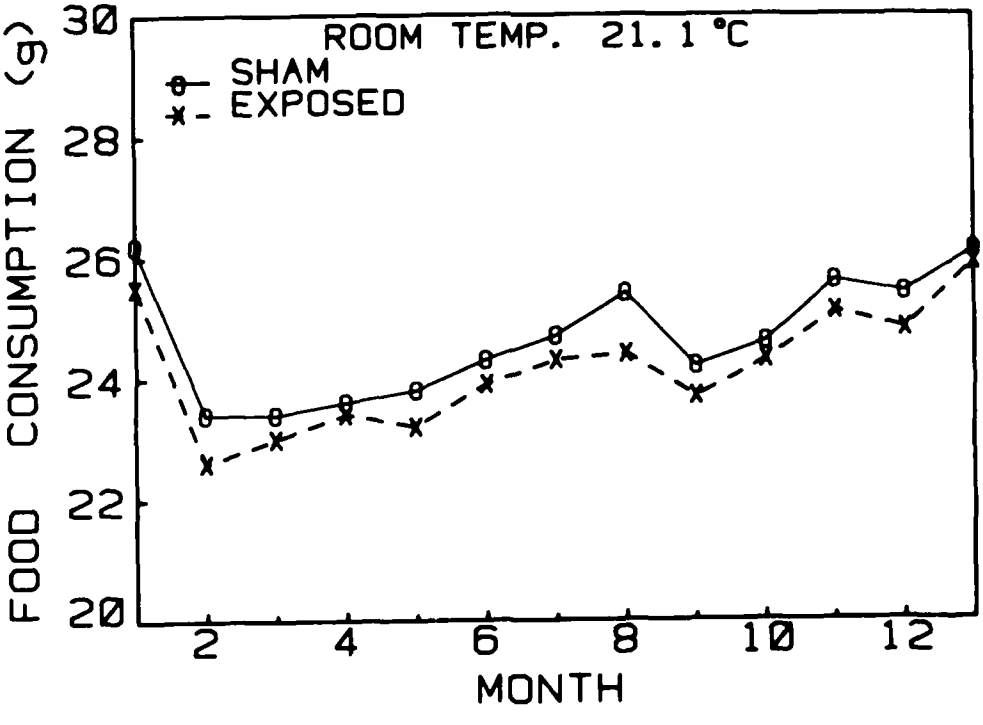

Figure 4. Mean food consumption of the 12-mo exposure.

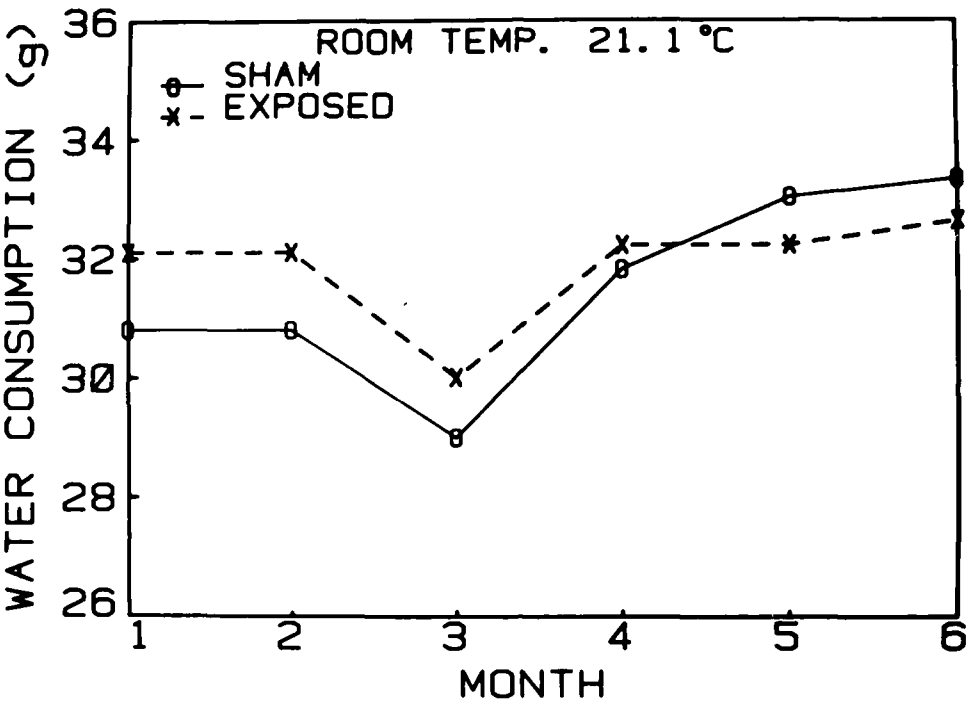

Figure 5. Mean water consumption of the 6-mo exposure.

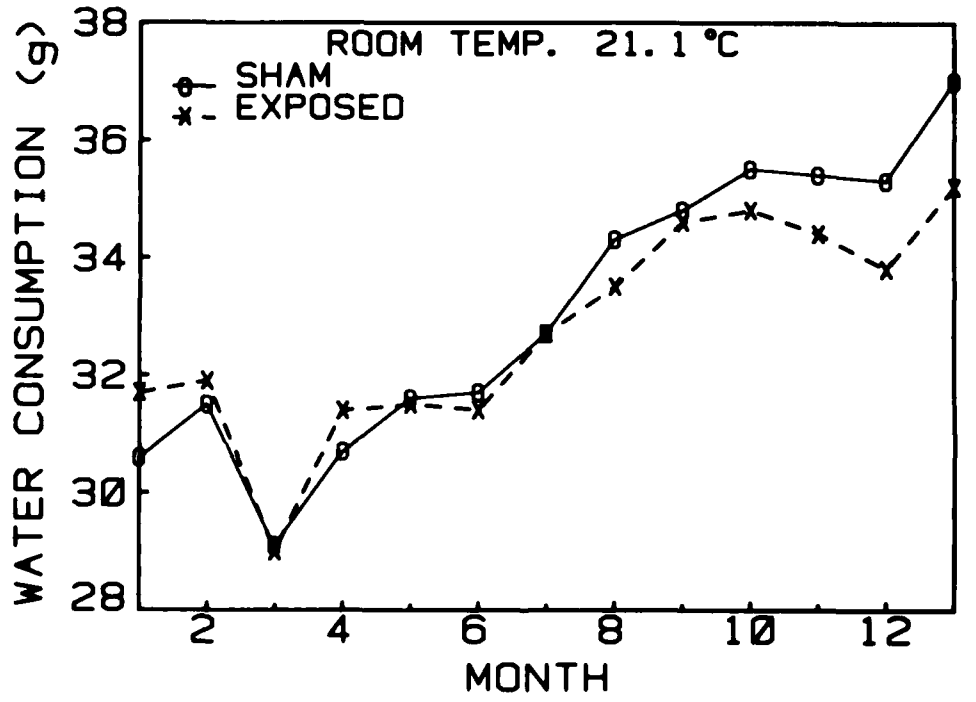

Figure 6. Mean water consumption of the 12-mo exposure.

Immune Competence Evaluation

Since the mid-1970s an increasing number of studies concerning the immunological response of various experimental animals to microwave irradiation have appeared in the scientific literature. This material provides a basic framework for inquiry concerning microwave irradiation but, when viewed as a whole, reveals inconsistencies and inadequacies that stimulate controversy about the significance of the basic biological findings and their role as a potential human-health hazard.

Increased lymphoid blastogenesis occurred after mitogen stimulation in mice exposed to 25-kHz electric fields (Bollinger et al., 1974) and in monkeys exposed to 27-MHz fields (Prince et al., 1972). Czerski (1974) reported increased numbers of peripheral lymphoblasts in exposed rats and mice; Huang et al. (1977, 1980) made the same observation. Studies by Liburdy (1979, 1980) indicate that both radiofrequency radiation (RFR) and steroid treatment affect lymphocyte circulation in mice. Liburdy speculates that the steroid release associated with thermal stress and the process of thermoregulation are significant factors responsible for RFR effects on the immune system. The studies of Smialowicz (1979) and Roszkowski et al. (1980) indicate that microwave-hyperthermia-evoked changes in hemopoiesis and lymphopoiesis can for the most part be attributed to nonspecific stress responses or to the direct thermal effect on immunocompetent cells. When studied in vitro, the metabolism of lymphocytes and reticuloendothelial cells is stimulated by hyperthermia at 38-40^{o}C and is dramatically inhibited, then irreversibly damaged, upon exceeding 43^{o}C (Roszkowski et al., 1979). Therefore, changes in lymphoid blastogenic reactivity to mitogens and synthesis of antibodies by lymphocytes in these in vitro studies seem to be influenced by microwave energy deposition. This may be the basis of the stimulatory effect of microwaves on the maturation of B-lymphocytes, leading to the reported increased presence of complement-receptor (CR^{+}) cells (Schlagel et al., 1980), increased mitogen response, and increased plaque-forming ability (antibody production) (Wiktor-Jedrzejczak et al., 1980; Sulek et al., 1980; Liddle et al., 1980).

The stimulatory effects observed in the cited studies have not been reproduced by others and exhibit discrepancies which may be explained by variations in level and duration of exposure, especially the studies performed at low-field intensities (Smialowicz, 1979, 1981; Huang, 1980; McRee et al., 1980; Hellstrom et al., 1981).

The stage of maturity of an animal's immune system may be a factor in determining susceptibility to modification by microwave irradiation. When immunological assessments were made at 3 and 6 wk of age, no consistent differences in plaque-forming ability, mitogen response, or natural killer (NK) cell activity were observed between irradiated and sham-irradiated mice exposed in utero to 2450-MHz microwaves at an incident power density of 28 mW/cm^2 for 100 min daily from day 6 to day 18 of pregnancy (Smialowicz et al., 1982b). These results contrast with alterations seen by Wiktor-Jedrezejak et al. (1977) after postnatal exposure of mice: A mild stimulatory effect on splenic lymphocytes was indicated by increased responsiveness to mitogens and increased antibody-forming spleen cells. In rats an enhanced lymphocyte responsiveness was found when they were exposed in utero at 425 MHz (Smialowicz et al., 1982a) and when irradiated in utero and during early postnatal life (Smialowicz, 1979; Smialowicz et al., 1982b). These studies suggest that only postnatal irradiation of mice may induce changes in lymphocyte responsiveness, whereas rats show increased lymphocyte responsiveness when exposed either in utero or postnatally. This variation between rats and mice may be related to species differences in maturation of the immune system.

Smialowicz et al. (1982a) suggest that exposure to microwave radiation tends to modify immunological competence in rats, resulting in an SAR in excess of their basal metabolic rate. Bowhill (1981) affirms that exposure to acute microwave irradiation or an exposure lasting from one to several days stimulates immune system activity. Bowhill further speculates that there is a biphasic reaction to the immune system after exposure. That supposition is not documented by experimental data, however, and is contrary to findings in long-term exposure experiments with mice (Bollinger et al., 1974), monkeys (Prince et al., 1972), and rats (Kunz et al., 1983).

During the most recent rat experiment conducted at the University of Washington, several immunological tests were performed on 10 exposed and 10 sham-exposed rats after a 1-yr exposure. The most significant finding was the enhanced splenic lymphocyte response to various mitogens and the absolute increase in splenic B and T cells of the exposed rats. That long-term study provided for a broad screening of immunological competence, including the evaluation of splenic lymphocytes for response to various B- and T-cell-specific mitogens as an in vitro measure of lymphoid cell functionality, DNA synthesis, and cell division. These included the plant lectins Con A and phytohemaglutinin (PHA) that specifically stimulate T-cell populations. The B-cell-specific mitogens bacterial lipopolysaccharide (LPS) of E. coli, PPD, and PWM were also used. Enumerations of B and T cell populations in the spleen were determined by using direct and indirect immunofluorescent techniques respectively.

Splenocyte populations were also assayed for frequency of complement-receptor positive cells. Spleen cells obtained from 10 exposed and 10 sham-exposed rats were assayed in duplicate for ability to form plaque-forming cells in response to sheep red-blood cells (SRBC), as a measurement of antibody-producing ability. One-half of each group was injected with SRBC, and the other half with phosphate-buffered saline (PBS) as a control. No significant differences were seen between the exposed and sham-exposed rats in the percentages of CRP cells in the spleens. In the animals immunized with SRBC there was a slight (statistically insignificant) increase in plaque per spleen for the exposed animals relative to the sham-exposed, indicating no major impairment of the antigen presentation system and no deficiency of the B-cells' ability to produce antibodies in the presence of functional T cells.

Relative to the sham-exposed group, the exposed animals had a significant increase in both splenic B and T cells. This is suggestive of a general stimulation of lymphopoiesis or of selective lymphoid-cell sequestration in the animals exposed to RFR. The mitogen stimulation studies revealed a significant difference in the patterns of response to various B- and T-cell-specific mitogens. The data indicate, using the multivariate

Hotelling T^2 statistic, that the exposed animals had a nonsignificant increase for PHA and a significant increase for LPS and PWM at the .05 level. The exposed animals showed a significantly increased response for Con A and a decreased response for PPD at the .01 level. The results of the mitogen test may indicate a selective effect on the lymphoid system by RFR, enabling the spleen cells to have a pronounced and selective mitogen response.

Our objectives were to extend the observations of the previous study and to use more sensitive methodologies with larger numbers of animals in order to obtain more reliable results. Specifically, we investigated the proliferative response in vitro of thymus cells, splenocytes, and bone marrow cells to a panel of polyclonal activators. Using flow cytometric techniques in combination with specific antibodies to T- and B-lymphocyte subpopulations, we determined the cellular composition of the thymus gland, spleen, and bone marrow. Finally, using the colony-forming assay in agar (CFU-C), we quantified hematopoietic progenitor cells of the monocyte/macrophage and granulocyte lineages in the bone marrow compartment.

Methods

Study Design and Method of Analysis. Eighty rats were randomly divided into two groups of 40 each. One group received microwave radiation; the other served as an unexposed control group. After the 6-mo exposure period, on each of 5 kill days eight rats were sacrificed and their spleen, thymus, and bone marrow cells assayed according to the following plan:

Kill Day	No. Exposed	No. Controls
1	5	3
2	6	2
3	2	6
4	4	4
5	3	5

After the 12-mo study, 40 rats were sacrificed and their organs removed and assayed on 4 kill days as follows:

Kill Day	No. Exposed	No. Controls
1	5	5
2	5	5
3	5	5
4	5	5

To accommodate the imbalance present in the two groups with respect to the random effect of "day," we used the General Mixed Model Analysis of Variance Program BMDP-3V to analyze these data (Table 1). The method of estimation was "maximum likelihood"; computations were done on a DEC-10 computer.

Exposure Facility and Dosimetry. The exposure facility consisted of 50 waveguides for active exposure and 50 for sham exposure. The active waveguides were powered by two 2450-MHz pulse-microwave generators, each capable of delivering a maximum of 10-W average power at 800 pps with a 10-μsec pulse width. This carrier was square-wave modulated at an 8-Hz rate. The power distribution system delivered 0.144 W to each exposure waveguide, for an average power density of .48 mW/cm^2. Whole-body calorimetry, thermographic analysis, and power-meter-output analysis indicated that these exposure conditions resulted in average SARs ranging from approximately 0.4 W/kg for a 200-g rat to 0.15 W/kg for an 800-g rat. Details of the exposure facility and dosimetry have been reported before (Guy et al., 1983a; Chou et al., 1984).

TABLE 1. STATISTICAL MODEL

$$Y_{ijk} = u + \alpha_i + b_j + e_{ijk} \qquad i = 1,2 \qquad j = 1...5$$

group (fixed) day (random) error (random)

assumptions: $b_j \sim N(0, \sigma_b^2)$

$e_{ijk} \sim N(0, \sigma_e^2)$

Test statistic for the null hypothesis of no group effect is the likelihood-ratio test of $H_o: \alpha_i = 0$; p-value is obtained by referencing the asymptotic distribution of this statistic, which is $\chi^2(1)$.

Mitogen Stimulation Studies. At the end of the 6- and 12-mo exposures, the animals were removed from the waveguide at approximately 1.5-min intervals, taken to the adjoining necropsy room, anesthetized, and exsanguinated. The spleen, thymus, and femur were removed aseptically, and an approximate 8% section of spleen and thymus was removed for histopathological analysis. The remaining portion of each spleen and thymus was delivered to the immunology laboratory for analysis within 15 min of removal.

Cell Preparations.

1. Single-cell suspensions of spleen and thymus were prepared by gentle teasing from the capsule and suspension in RPMI 1640 medium.
2. Bone-marrow cells were flushed from femoral cavities with cold medium. A single-cell suspension was made by repeated aspiration in a pipette. Erythrocyte lysis was accompanied by treatment of the cell suspension with 0.83% buffered ammonium chloride.

Colony-Forming Unit-Culture. Our laboratory used a modified method of Bradley and Metcalf (1969) to detect CFU-C. The cell suspension of marrow was mixed with 0.3% Bacto-agar in MEM-alpha medium containing 25% heat-inactivated pooled human serum. Other culture additives included glutamine, antibiotics, 1% bovine serum albumin, 2-ME, and 10% PWM spleen-conditioned medium. The conditioned medium served as a source of colony-stimulating activity (CSA). The cells were added to give a final concentration of 1×10^5 cells per ml of agar, and 1 ml was placed in each 35- x 10-mm petri dish to gel (Falcon Plastics, Oxnard, CA). After the gel stage, the petri dishes were incubated in a CO_2 incubator at 37°C with 5% CO_2 to 95% air. After 7 days, the numbers of colonies (clusters of over 50

cells) were counted using an inverted microscope. The means and standard deviation of triplicate plates were calculated for each experimental condition.

Cytochemical Examination of Cells Grown in Agar Gel. All colonies in an agar gel can be stained cytochemically to detect the presence of chloracyl esterase. Granulocyte and macrophage clusters of colonies can be easily distinguished from one another by the production of reaction products from naphthol AS-D chloroacetate. After the colonies had been counted, the agar medium was transferred from the petri dishes to glass slides. To absorb the liquid, Whatman #1 filter paper was placed on the surface. This process produced a flattened, dried preparation, ready for fixation in 10% cold neutral buffered formalin for 10 min. Fixed preparations were then washed in water, dried, and incubated for 45 min at 37°C in freshly prepared substrate. The slides were washed in water, air-dried, and counterstained with hematoxylin. We examined the slides under a low-power microscope to count total number of colonies and the number with all cells containing red-reaction production (neutrophil), the number without product (macrophage), and the number in which some cells contain reaction product (mixed neutrophil-macrophage). The data were expressed as the fraction of total colonies that are macrophage, neutrophil-macrophage, and neutrophil. The number of CFU-C that belong to each of the categories could also be calculated. From these data, shifts in the frequencies and number of progenitors, granulocyte, granulocyte-macrophage, and macrophage in the tissue could be detected. Table 2 details the variables analyzed.

TABLE 2. CFU-C AND MITOGEN STIMULATION VARIABLES ANALYZED IN STUDY

A. CFU-C total and differential counts

1. Total colonies
2. Macrophage colony count
3. Granulocyte colony count
4. Mixed colony count

B. Mitogen stimulation assay

Differences in observed scale of ^{125}IUdR cpm
(stimulated response - control;
e.g., Con A - medium control)

To compare stimulation indices in previous study, we also analyzed differences in natural log scale
[ln(stimulated-response/control);
e.g., ln(Con A/medium control)]

1. For thymus

Con A
PHA
PWM
medium control

2. For spleen

LPS
PHA
PPD
Con A
PWM

Separate likelihood-ratio tests were performed on each variable to test H_o: α_i=0 (at 0.05 level).

Measurement of DNA Synthesis. Aliquots of 0.1 ml of cell suspensions placed into a 96-well flat-bottomed microtiter plate (Linbro-Flow Labs, Inglewood, CA) were cultured and the DNA synthesis activity measured. After the appropriate culture period, cells were incubated with 5.0 μCi/ml of ^{125}I-iododeoxyuridine (^{125}IUdR) (2000 Ci/mmol, specific activity; New England Nuclear) and 10^{-6}M 5-fluorodeoxyuridine (Aldrich Chemical Co., Milwaukee, WI) for 4 h and then harvested by an automated cell harvester. The radiolabel incorporation was determined by a gamma counter. The data were expressed as net counts per minute (experimental cpm - control cpm) and as a stimulation index (E/C). Table 2, Section B, shows the variables analyzed.

B- and T-Cell Enumeration. Spleen cell, bone marrow, and thymocyte suspensions were prepared for each animal, as previously described for the mitogen assays. Then, 2 x 10^6 cells of each tissue were pelleted in 1-ml microfuge tubes and stained for the detection of either B or T cells as follows:

B cells -- 50 μl of fluorescein isothiocyanate (FITC) $(Fab')_2$ goat antirat Ig (polyvalent) antibody was added to the cell suspensions and incubated on ice for 30 min

T cells -- a proper volume of FITC monoclonal mouse anti-Thy 1.1 antibody was added to cell suspensions and incubated on ice for 30 min

The cells were then suspended in media, centrifuged to remove the excess FITC antibody, and resuspended in media containing 0.1% sodium azide. Samples were either analyzed in an orthocytofluorograph or fixed with 2% paraformaldehyde for analysis at a later time. Table 3 lists the variables analyzed.

TABLE 3. B- AND T-CELL VARIABLES ANALYZED IN STUDY

Quantitation of B and T cells by expression of surface immunoglobulin and Thy 1.1 antigens, respectively, and mean population intensity of each antigen per cell basis:

1. Thymocytes and splenocytes

   a. Percentage of all viable cells expressing cell-surface antigens
   b. Percentage of low light scatter expressing cell-surface antigens
   c. Percentage of high light scatter expressing cell-surface antigens

2. Bone marrow cells

   a. Percentage of all viable cells expressing cell-surface antigens
   b. Percentage of low light scatter expressing cell-surface antigens
   c. Percentage of high light scatter expressing cell-surface antigens
   d. Percentage of medium light scatter expressing cell-surface antigens

Quantitation of cell populations in each tissue using correlated forward-angle light scatter (cell size) and right-angle light scatter (cellular complexity):

1. Thymocytes and splenocytes

   a. Total cells per organ x 10^{-6}
   b. Percentage of total cells in low-light-scatter population
   c. Percentage of total cells in high-light-scatter population

2. Bone marrow cells

   a. Total cells per organ x 10^{-6}
   b. Percentage of total cells in low-light-scatter population
   c. Percentage of total cells in high-light-scatter population
   d. Percentage of total cells in medium-light-scatter population

Separate likelihood-ratio tests were performed on each variable to test H_0: $\alpha_i=0$ (at 0.05 level).

Orthocytofluorograph Analysis. By quantitative fluorescence the various cell populations were measured with an Ortho System 50H Cell Sorter and Ortho model 2150 computer and analysis unit (Ortho Diagnostic Systems, Westwood, MA). The instrument is equipped with dual argon and krypton ion high-power lasers for optimum two-color [e.g., fluorescein isothiocyanate (FITC) and rhodamine isothiocyanate (RITC) fluorescence] analysis. The computer unit allows complex gating analysis of any combination of up to four available parameters; for example, forward- and right-angle scatter and green and red fluorescence. Data are stored on 5-Mbyte hard disks allowing later display and reanalysis. As illustrated in Figure 7, correlated forward-angle light scatter (cell size, y axis) and right-angle light scatter (complexity of cell structure, x axis) was used to select cell populations from each tissue to be assayed for B- and T-cell specific markers respectively.

For example, in Figure 7 the rectangle (region 1) encompasses all intact cells in each tissue. Regions 2 and 3 are low- and high-light-scatter populations respectively. In the case of bone marrow (BM), region 4 includes cells with medium forward scatter and high right-angle-scatter (e.g., granulocytes), hereafter referred to as the "medium-light-scatter population."

Figure 8 depicts the fluorescence histograms of splenocyte and thymocyte populations. In panels A and B, aliquots of splenocytes were exposed to FITC-goat antidog C3 (negative control) and to FITC-goat antirat IgG respectively. Regions (channels 80-200) were determined where 5% of the nonspecifically stained cells (panel A) were included in the B-cell population (panel B). Panels D and C illustrate the T-cell stain, FITC Thy 1.1, on thymocyte suspensions and its control respectively.

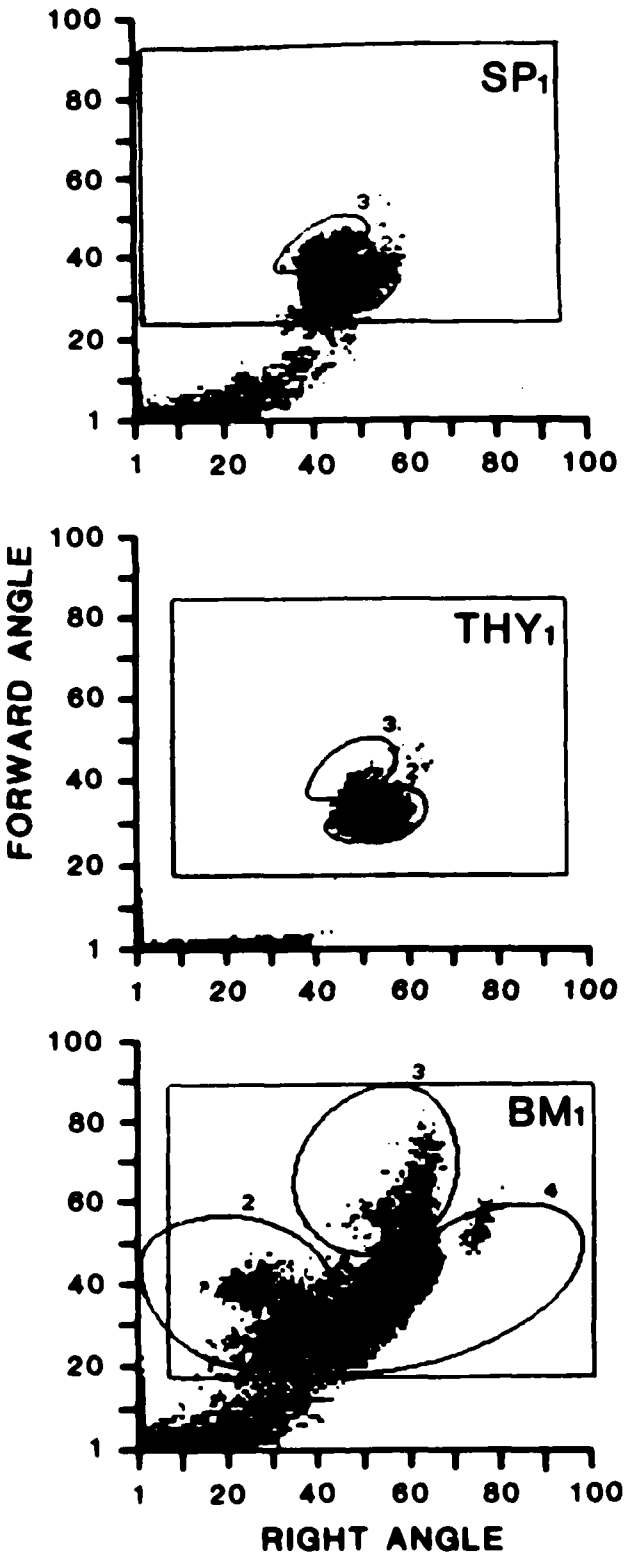

Figure 7. Scatter histograms from the orthocytofluorograph 50H.

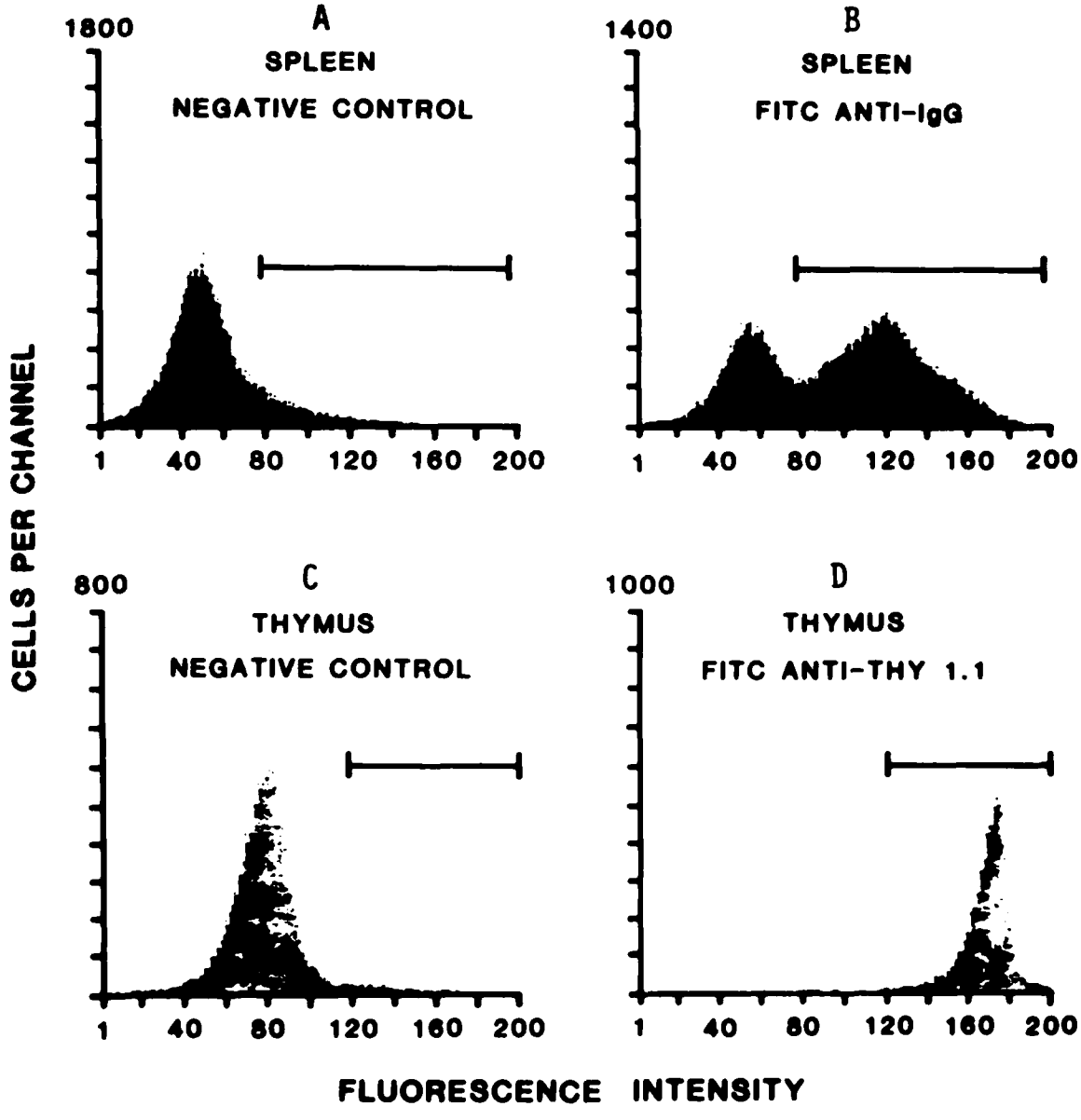

Figure 8. Fluoresence histograms of splenocyte and thymocyte populations.

Results

CFU-C Assay. The numbers of hematopoietic progenitor cells were quantitated in the femurs of exposed and sham-exposed rats, using the method of Bradley and Metcalf (1969). As shown in Figure 9, the data demonstrated that rats exposed for 6 mo had significantly elevated numbers of total ($p = 0.014$), macrophage ($p = 0.046$), and granulocyte colonies ($p = 0.016$); no differences were observed in the numbers of mixed colonies. As depicted in Figure 10, similar results were observed after exposure for 12 mo: exposed rats had significantly elevated numbers of total ($p < 0.001$), macrophage ($p < 0.001$), and granulocyte colonies ($p < 0.079$). After both 6- and 12-mo exposures, the cellularity of the femoral cavities was not different between experimental and control animals.

Mitogen Responses. To detect functional alterations induced by RFR irradiation in subpopulations of lymphoid cells from the spleen and thymus, we cultured single-cell suspensions of each organ in vitro with the optimal concentration of mitogens and assayed for DNA synthesis on the peak day of DNA synthesis response (preliminary data, not shown). All DNA synthesis responses were analyzed both in the observed scale and the natural logarithmic (ln) scale. Differences in the observed scale (stimulated - control) reflect excess stimulated response over baseline in counts per minute. Differences in the ln scale [ln(stimulated) - ln(control) = ln(stimulated/control)] are ln-transformed stimulation indices; the ln transformation decreases the influence of large outliers. No significant differences between exposed and sham-exposed rats were observed in the proliferation of thymocytes to Con A, PHA, and PWM (Table 4) after 6- and 12-mo of exposures. This result was unchanged when rats with outlying values were excluded from the analysis.

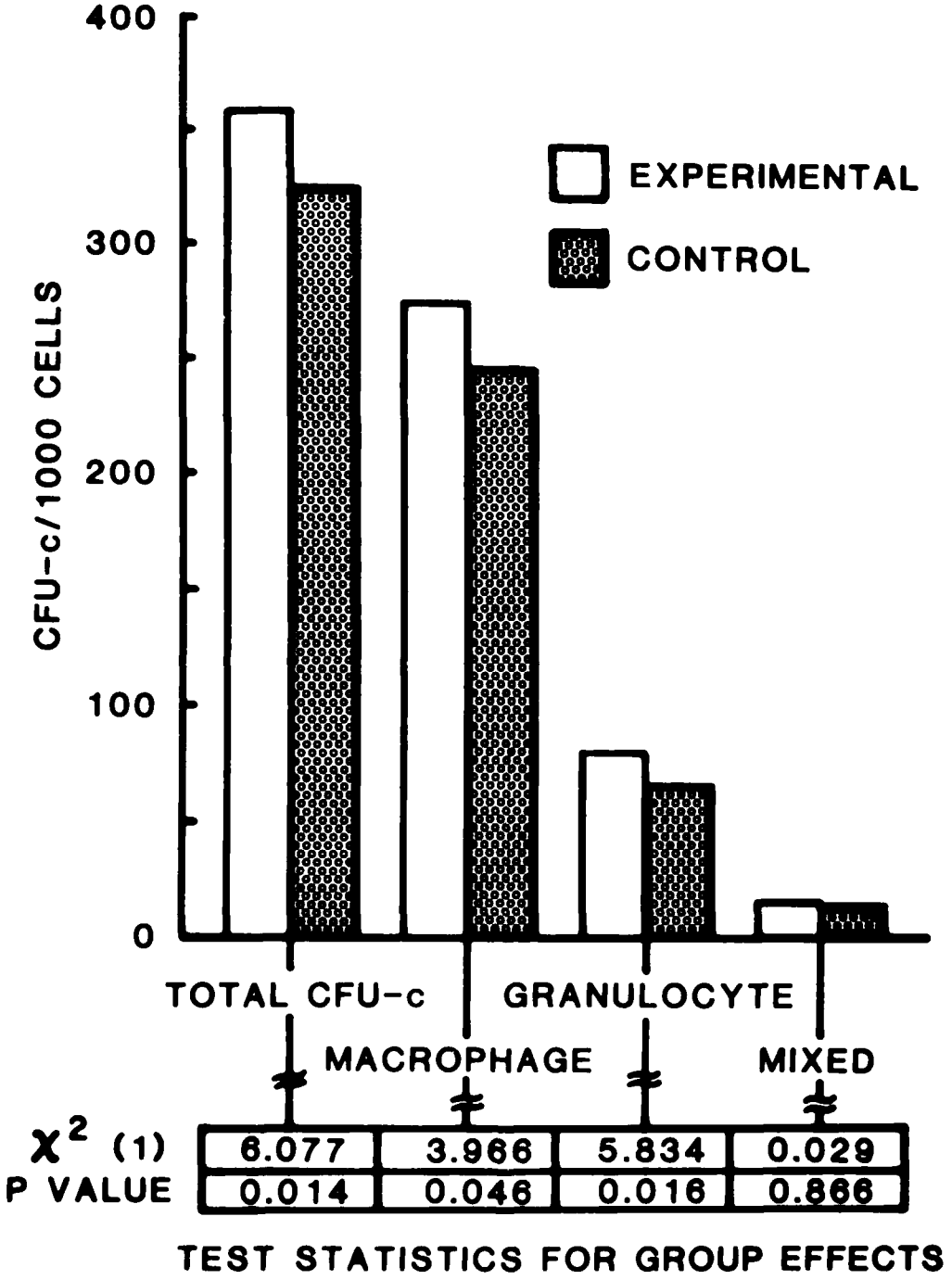

Figure 9. Effects of 6-mo exposure on the levels of total, macrophage, granulocyte, and mixed CFU colonies.

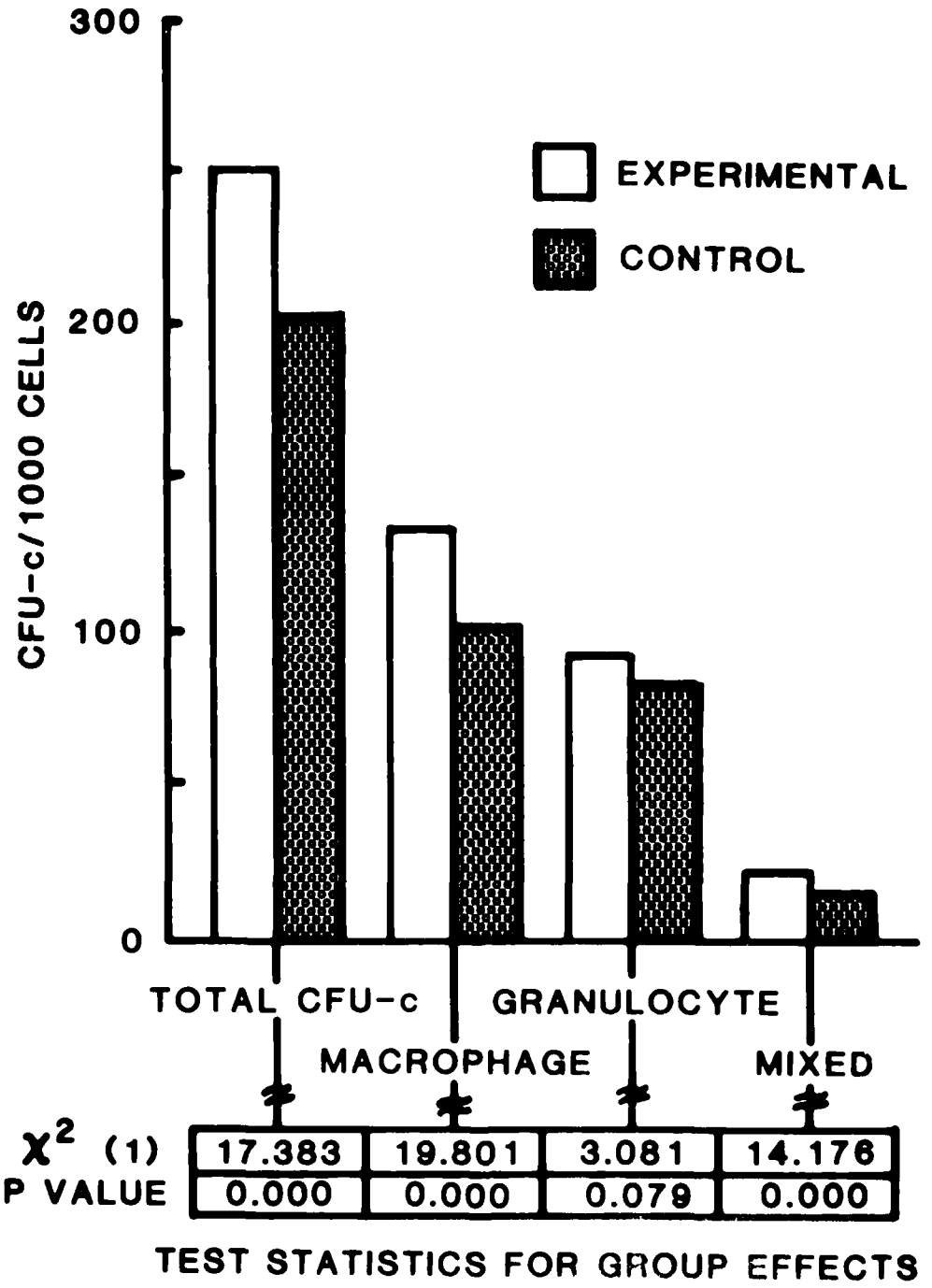

Figure 10. Effects of 12-mo exposure on the levels of total, macrophage, granulocyte, and mixed CFU colonies.

Test statistics for group differences with respect to DNA synthesis response of splenocytes to a panel of mitogens are given in Table 5. No significant group differences were observed for the mitogens at the .05 level. The LPS and PPD results were somewhat sensitive to data anomalies mentioned in the discussion. Analysis of the DNA synthesis response of splenocytes after 12 mo of exposure revealed no significant differences between experimental and control animals.

TABLE 4. ANALYSIS OF PROLIFERATIVE RESPONSE OF THYMOCYTES IN VITRO TO PHA, PWM, AND CON A

Exposure period (mo)	Culture stimulant	Mean ^{125}IUdR cpm (standard error)		Chi-squared (1) (p value)
		Exposed	Unexposed	Observed scale
6	Con A[a]	43,332 (7,176)	33,957 (4,918)	3.051 (0.081)
6	PHA[a]	3,507 (2,055)	809 (89)	1.771 (0.183)
6	PWM[a]	584 (248)	224 (43)	2.131 (0.144)
12	Con A[a]	91,122 (10,927)	87,913 (14,748)	0.068 (0.794)
12	PHA[b]	2,055 (371)	2,166 (674)	0.023 (0.879)
12	PWM[c]	1,492 (251)	1,778 (398)	0.389 (0.533)

a: n = 40
b: n = 29
c: n = 38

TABLE 5. ANALYSIS OF RESPONSES OF SPLENOCYTES IN VITRO FROM IRRADIATED AND CONTROL ANIMALS TO LPS, PHA, PPD, CON A, AND PWM

Exposure period (mo)	Culture stimulant	Mean ^{125}IUdR cpm (standard error)		Chi-squared (1) (p value)
		Exposed	Unexposed	Observed scale
6	LPS[a]	16,610 (3,252)	18,039 (3,174)	2.813 (0.094)
6	PHA[a]	29,913 (6,258)	28,997 (4,441)	0 (0.983)
6	PPD[a]	2,592 (570)	2,446 (421)	1.264 (0.264)
6	Con A[a]	41,545 (7,199)	50,730 (6,689)	0.046) (0.830)
6	PWM[a]	21,815 (4,300)	26,093 (3,766)	1.337 (0.248)
12	LPS[b]	16,504 (2,572)	12,428 (2,688)	2.490 (0.115)
12	PHA[c]	8,678 (2,230)	10,141 (4,414)	0.122 (0.727)
12	PPD[d]	184 (86)	206 (72)	0.042 (0.838)
12	Con A[a]	129,723 (21,570)	110,942 (20,896)	1.075 (0.300)
12	PWM[a]	4,528 (1,092)	4,571 (1,804)	0.0 (0.983)

a: n = 40
b: n = 39
c: n = 38
d: n = 30

Quantitation of T and B Cells by Flow Cytometric Techniques. Thymocyte suspensions prepared from exposed and sham-exposed rats for 6- and 12-mo groups were each subsequently stained with FITC goat antirat IgG (B cells) and FITC anti-Thy 1.1 (T cells). The cells were analyzed on the orthocyto-fluorograph for six parameters (Table 6). No significant differences between groups were observed in the percentage of B (s-Ig) or T (Thy 1.1) cells in the thymii. The population mean expression of s-Ig and Thy 1.1 cell-surface antigen did not differ significantly between exposed and sham-exposed animals. Due to technical difficulties with the immuno-chemical staining on the fifth day of the experiment, three experimental and five control animals were excluded from the analysis of the 6-mo study. Animals exposed for 12 mo demonstrated marginally significant reduction in the percentage of B cells in the entire thymus gland and, as expected, reduction in the fraction of cells with low light scatter that carry s-Ig. In addition, the mean expression of Thy 1.1 antigen was reduced ($p = 0.029$) in cells whose light scatter is consistent with that of small lymphocytes.

As depicted in Table 7, analysis of marrow and spleen cells from exposed and sham-exposed rats for the presence of B and T cells revealed the following: (a) no evidence of group differences in the percentage of B and T cells and in the mean expression of s-Ig and Thy 1.1 (or B or T cells); (b) marginally significant group differences ($p = 0.05$) on the percentage of total viable B cells in the marrow, where the exposed animals had fewer B cells in the marrow; (c) no other statistically significant group differences. After 12 mo of RFR exposure, the T-cell population in the marrow was highly significant, whereas the mean surface density of s-Ig was significantly reduced in both the low- and high-light-scatter spleen-cell population. No other group differences were observed (see Table 8).

In Table 9, the analysis of correlated light-scatter parameters revealed that exposed animals had significantly fewer marrow cells with low-light-scatter characteristics than did sham-exposed rats. No group differences with respect to the other light-scatter characteristics were evident for either 6 or 12 mo of RFR exposure.

TABLE 6. ANALYSIS OF CELLULAR COMPOSITION IN THYMUS GLAND OF IRRADIATED AND CONTROL ANIMALS BY FLOW CYTOMETRY AND SPECIFIC ANTIBODIES DEFINING B AND T LYMPHOCYTES

	Chi-squared (1) (p value)			
	6 Months		12 Months	
Variable analyzed	B cells	T cells	B cells	T cells
Percentage of entire population bearing antigen	2.042 (0.153)	3.007 (0.083)	3.740 (0.053)	0.738 (0.390)
Percentage of low light scatter bearing antigen	0.061 (0.805)	1.680 (0.195)	3.996 (0.046)	0.851 (0.356)
Percentage of high light scatter bearing antigen	0.351 (0.533)	0.550 (0.458)	ND	ND
Mean expression of antigen over entire population	0.309 (0.578)	1.781 (0.182)	0.024 (0.877)	3.358 (0.067)
Mean expression of antigen in low-light-scatter population	0.159 (0.691)	0.066 (0.797)	0.198 (0.656)	4.761 (0.029)
Mean expression of antigen in high-light-scatter population	0.258 (0.611)	1.961 (0.161)	ND	ND

TABLE 7. ANALYSIS OF CELLULAR COMPOSITION IN BONE MARROW AND SPLEEN OF IRRADIATED AND CONTROL ANIMALS BY FLOW CYTOMETRY AND SPECIFIC ANTIBODIES DEFINING B AND T LYMPHOCYTES

	Chi-squared (1) (p value) 6 Months			
	B Cells		T Cells	
Variable analyzed	Marrow	Spleen	Marrow	Spleen
Percentage of entire population bearing antigen	3.831 (0.050)	0.945 (0.331)	0.900 (0.343)	1.229 (0.268)
Percentage of low light scatter bearing antigen	1.972 (0.160)	0.730 (0.393)	0.307 (0.579)	0.982 (0.322)
Percentage of medium light scatter bearing antigen	3.551 (0.060)	ND	0.969 (0.325)	ND
Percentage of high light scatter bearing antigen	1.468 (0.226)	0.823 (0.364)	2.443 (0.118)	0.739 (0.390)
Mean expression of antigen over entire population	0.189 (0.664)	0.012 (0.913)	0.043 (0.835)	1.537 (0.215)
Mean expression of antigen in low-light-scatter population	0.006 (0.938)	0.852 (0.356)	0.191 (0.662)	0.094 (0.759)
Mean expression of antigen in medium-light-scatter population	0.530 (0.467)	ND	0.758 (0.384)	ND
Mean expression of antigen in high-light-scatter population	2.257 (0.133)	0.000 (0.996)	0.655 (0.418)	1.925 (0.165)

TABLE 8. ANALYSIS OF CELLULAR COMPOSITION IN BONE MARROW AND SPLEEN OF IRRADIATED AND CONTROL ANIMALS BY FLOW CYTOMETRY AND SPECIFIC ANTIBODIES DEFINING B AND T LYMPHOCYTES

	Chi-squared (1) (p value) 12 Months			
	B Cells		T Cells	
Variable analyzed	Marrow	Spleen	Marrow	Spleen
Percentage of entire population bearing antigen	1.782 (0.182)	0.633 (0.426)	4.248 (0.039)	0.098 (0.754)
Percentage of low light scatter bearing antigen	0.578 (0.447)	0.081 (0.775)	1.337 (0.248)	0.023 (0.879)
Percentage of high light scatter bearing antigen	0.921 (0.337)	3.755 (0.053)	0.613 (0.434)	1.485 (0.223)
Mean expression of antigen over entire population	0.400 (0.527)	2.996 (0.083)	1.537 (0.215)	0.215 (0.643)
Mean expression of antigen in low-light-scatter population	0.395 (0.530)	5.684 (0.017)	2.208 (0.137)	0.198 (0.656)
Mean expression of antigen in high-light-scatter population	0.411 (0.522)	17.134 (0.000)	0.620 (0.431)	1.319 (0.251)

TABLE 9. FLOW CYTOMETRIC ANALYSIS OF CELLULAR COMPOSITION OF BONE MARROW, SPLEEN, AND THYMUS OF IRRADIATED AND CONTROL ANIMALS, USING FORWARD-ANGLE (CELL SIZE) AND RIGHT-ANGLE (CELL COMPLEXITY) PARAMETERS

	Chi-squared (1) (p value)					
	6 Months			12 Months		
Variable analyzed	Marrow	Spleen	Thymus	Marrow	Spleen	Thymus
Total nucleated cells per organ x 10^6	0.032 (0.857)	0.232 (0.630)	1.377 (0.241)	ND	ND	ND
Cell count per mg x 10^6	ND (0.739)	0.111 (0.358)	0.844	ND (0.675)	0.175 (0.236)	1.403
Percentage of cells with low light scatter	5.522 (0.019)	0.017 (0.896)	0.380 (0.537)	1.549 (0.213)	0.218 (0.641)	0.122 (0.727)
Percentage of cells with medium light scatter	2.673 (0.102)	ND	ND	ND	ND	ND
Percentage of cells with high light scatter	2.263 (0.133)	0.541 (0.462)	0.009 (0.923)	1.961 (0.161)	0.083 (0.773)	ND

Discussion

Significant issues dealing with the effects of low-level chronic exposure of laboratory animals to RF radiation have remained unresolved. Alterations in the hematopoietic and immunologic networks have been reported in animals exposed to RF at and below 10 mW/cm^2, but these effects could be due to thermal changes within the animals. The experiments reported here demonstrate alterations in the hematopoietic and immunologic systems of rats after long-term (6 and 12 mo) exposures to very low levels of RFR (SAR, 0.15-0.4 W/kg; .48 mW/cm^2). Effects of the 6-mo exposure include (a) an increased number of marrow hematopoietic progenitor cells (CFU-C); (b) increased proliferative responses to PPD by splenic B cells; (c) decreased total B lymphocytes in the marrow; (d) decreased proportion of low light scatter, i.e., small cells in marrow. The following effects are associated with the 12-mo exposure to RFR: (a) increased number of hematopoietic precursors; (b) reduction in cell-surface expression of Thy 1.1 surface antigen on thymocytes; (c) reduction in the mean cell-surface density of s-Ig on small lymphocytes (low light scatter) in the spleen. Some of these seven significant group differences may be statistical artifacts since 70 separate tests were done at the .05 level on animals exposed to RFR for either 6 or 12 mo. Even if the null hypothesis of no group difference were true, we would expect to find, on the average, 3.5 statistically significant differences after both the 6- and 12-mo exposures.

In contrast to a previous study at this institution, among the rats exposed for 6 mo we observed marginally increased spleen cell response to the B-cell mitogen PPD and no increased proliferative response to LPS, PWM, or Con A. Relative to days 2-5, PPD control responses were extremely low on day 1 for both exposed and nonexposed rats. Stimulated values for day 1 were within the range of values for assays on the other days. The ln (stimulation index) ratio is sensitive to this type of data anomaly. When day 1 was excluded from analysis, the mean responses for the two groups were marginally significantly different in the opposite direction; i.e., the mean ln (PPD/PPD control) response of the exposed group was lower than that of the nonexposed ($X^2(1) = 3.371$, $p = .066$). Since the PPD response showed no significant differences between the groups in the observed scale,

either including or excluding day 1, we believe this particular result may be spurious. In the LPS assay, stimulated responses were actually lower than the medium control values for one exposed rat assayed on day 2 and three nonexposed rats assayed on day 3. When those four observations were excluded from analysis, (1) the exposed animals exhibited a significantly lower response to LPS in the observed scale than did the nonexposed (X^2 = 4.637, p = 0.31), and (2) no significant difference was observed for LPS in the ln scale.

In contrast to our previous study where microscopic quantitation of T and B cells in the spleens of exposed and sham-exposed rats was used, the flow cytometric approach (where 10,000 cells were evaluated) revealed no group alterations in the number and frequency of T or B cells. However, the mean expression of s-Ig was reduced on splenic B cells after 12 mo of RFR exposure.

Six months of exposure (but not 12 mo) produced a selective decrease in B cells in the marrow of exposed rats. Several explanations for such a change are possible: (1) the rate of primary B-cell production in the marrow may be decreased due to the decreased cycling of and/or numbers of B-cell precursors; (b) the rate of recirculation of B cells to the marrow may be lower; or (c) the release of newly born B cells from the marrow to the blood may be faster. Our data do not permit us to select one interpretation at this time. At the same time, the frequency of small (low light scatter) cells in the marrow of exposed rats was diminished. Low-light-scatter cells encompass small lymphoid cells and immature erythroid elements and noncycling stem cells.

The most surprising and consistent finding of both exposure periods was the increased number of hematopoietic progenitor cells (CFL-C) in the marrow of exposed rats. Increased CFU-C can be explained by a decreased survival of mature monocytes, macrophages, and granulocytes in the peripheral lymphoid tissues, which necessitates a compensatory increase in their progenitor-cell activity. Alternatively, RFR may have a direct effect on the progenitor cells, increasing their production rate. Finally, RFR may cause increased production of growth stimulatory hormones by macrophages, lung cells, and T cells; such hormones may then expand the pool of hematopoietic progenitors in the marrow.

Animal Health Profile

Many research projects to assess the biological effects and health hazards of microwave radiation have involved single or limited biological endpoint evaluations. During the last few years a health profile has proven more useful to researchers for diagnosing and understanding abnormalities in their experimental animals. Several advantages are inherent in the health profile evaluation of experimental rats in this study. The profile helps to uncover unsuspected organ-system malfunction. In animals with subclinical or undiagnosed abnormalities, the profile can aid in selecting appropriate tests to define the animal's problem. This allows emphasis to be placed on the current interpretation of profile results and the interrelation of different test results rather than on individual test selection. The health profile permits a better understanding of the pathophysiology of abnormal or disease states. As an organ system abnormality or disease changes, the blood chemistry and hematology can be monitored. The profile can demonstrate multisystemic organ involvement, often missed if only individual tests are performed. The profile test results may indicate a specific condition that requires other tests to assess the full significance of a particular abnormality.

During the exposure period the rats were bled every 6 wk, with the first bleeding during the 7th wk of exposure. This duplicates the experimental stress that animals in the original project were subjected to. The retro-orbital technique was used for bloodletting. At the first bleeding session 100 blood samples were tested for the corticosterone level. The serum collected from all other periodic bleedings was not analyzed other than to be retained as a pooled control for the serum chemistry evaluations. Hematological, corticosterone, thyroxine (T_4), protein electrophoresis, and serum chemistry evaluations were made on blood collected during the interim sacrifices at 6 and 12 mo of exposure.

The serum chemistry evaluation consists of a panel of 24 parameters from each sample, incuding various electrolytes, enzymes, and blood gases. This panel provides a multiphasic evaluation of the animals' physiological systems.

Although not a method for determining specific proteins, serum protein electrophoresis is considered valuable in organ panels or health profiles. It is the single most sensitive procedure for detecting monoclonal gammopathies and is used to determine certain proteins such as albumin and immunoglobulins. With a well-resolved system, even small monoclonal or oligoclonal bands can be easily identified, giving evidence of intense immunologic stimulation such as may accompany serious viral infections or tissue necrosis.

The hematological data are seldom diagnostic as individual tests but are invaluable as an adjunct to the health profile. They indicate the presence or absence of systemic stress, inflammatory disease, bone marrow disease, and neoplasia. This information may help in formulating a presumptive or final diagnosis or in monitoring a physiological or disease abnormality that may affect the immunological status of the animal and lead to invalid immunological test results.

Pituitary-adrenal axis activity as indexed by plasma corticosterone levels has long been interpreted as an indicator of general arousal, i.e., anxiety, fear, or stress. Stress can be attributed to very specific, noxious stimuli or to a diffuse, nonspecific stimulus complex, either physical or psychological in nature (Selye, 1950; Levine, 1972). Exposure to microwave radiation under various frequencies and power levels produces alterations in pituitary-adrenal activity (Lu et al., 1980), albeit at levels substantially higher than used in this study.

The assessment of plasma corticosterone was originally intended solely as a check on the stress-free nature of the experimental housing and protocol in compliance with the original statement of work. Of concern was the possibility that a stressful environment might result in artificial microwave effects or mask important microwave bioeffects. This endpoint also provides additional information about animal well-being prior to assessment of immunological competence.

Methods

Serum Chemistries. The blood for the serum chemistries was collected in B & D microtainer serum-separator tubes. The serum was separated and then refrigerated in separate glass vials until it was analyzed later that day on a Technicon SMAC computer-controlled biochemical analyzer. The analysis done on the SMAC analyzer for this project was directly supervised by a board-certified veterinary pathologist. The following test values were obtained by the SMAC analyzer:

1. Glucose
2. Urea nitrogen (BUN)
3. Creatinine
4. Sodium
5. Potassium
6. Chloride
7. Carbon dioxide
8. Uric acid
9. Total bilirubin
10. Direct bilirubin
11. Ionized calcium
12. Calcium
13. Phosphorus
14. Alkaline phosphatase
15. Lactate dehydrogenase (LDH)
16. Serum glutamic-oxaloacetic transaminase (SGOT)
17. Serum glutamic-pyruvic transaminase (SGPT)
18. Cholesterol
19. Triglycerides
20. Total protein
21. Albumin
22. Globulin

Thyroxine. The radioimmunoassay for T_4 is extremely sensitive, and small quantities of serum are required for the test. In the test procedure, serum of the 20 rats was mixed with radioactivity-labeled thyroxine (^{125}I) and 8 anilinolnapthalene sulfonic acid (ANS), then an immobilized T_4 antiserum was added and the mixture was incubated at room temperature. The ANS displaces the T_4 from the serum proteins. During incubation the displaced T_4 competes with the labeled T_4 for the immobilized T_4 antibodies on the basis of their relative concentrations.

The amount of labeled T_4 that binds with the antibody is inversely related to the amount of unlabeled endogenous T_4 present in the serum. After incubation the mixture was centrifuged and the immobilized T_4-antibody complex was concentrated at the bottom of the tube in the form of a pellet. The unbound T_4 in the supernatant was decanted, and the radioactivity associated with the pellet was counted. A standard curve prepared with precalibrated T_4 standards was used to determine the concentration of T_4 in the rat serum.

The procedure was a modified quantimmune T_4 radioimmunoassay (RIA) method based on the principles of RIA described by Berson and Yalow (1960). A Packard autogamma scintillation spectrometer 5130 was used.

Protein Electrophoresis. A Sepratek system (Gelman Instrument Co., Ann Arbor, MI) was used in the protein electrophoresis analysis. Eight serum samples (0.25 µl each) were applied with the eight-place applicator to a single cellulose acetate membrane (5.7 x 12.7 mm). Three of these membranes were mounted in a single electrophoresis chamber containing 700 ml buffer (tris-barbital-sodium barbital pH 8.8). The samples were electrophoresed for 60 min at 300 V, then the membranes were stained with Ponceau S solution, clarified, and mounted on glass slides. This produced well-defined serum protein fractions on a transparent background. Quantitation with a digital computing densitometer provided a scan of the optical density at 525 nm and computed the area under the portions of the scan corresponding to the various protein fractions.

Included in each batch of samples were several control serums. These consisted of commercially obtained pure albumin, beta globulin, and gamma globulin dissolved in saline. These controls assisted in locating the various fractions on the sample densitometer scan and provided a way to check the integration accuracy of the densitometer.

Hematology. The hematological tests followed standard laboratory practice. The hematological parameters, determined on a minimum of 0.3 ml of blood in EDTA, were

Hemoglobin	Packed cell volume (PCV)
White blood count (WBC)	Indices
Red blood count (RBC)	Differential count

A Coulter counter was used for the WBC and RBC; the hemoglobin was determined by the cyanomethemoglobin method, and trained hematology technicians did the differential counts. The blood film was prepared on a slide from a thoroughly mixed sample, and the slide was stained by Wright's method and evaluated microscopically. The work of the hematology technicians was supervised and reviewed by a board-certified veterinary pathologist. The data were compared with reference data from the literature and from the Laboratory Animal Data Bank.

Corticosterone Assay. The microfluorometric assay procedure used to analyze plasma corticosterone was based on a procedure described by Glick et al. (1964), and modified by Riley and Spackman (1976), for use on small samples (50 µl) obtained from mice. The technique was further modified to fit the needs of the original RFR exposure study on rats, using the retro-orbital bleeding technique. After correction for the presence of nonspecific fluorogens, the method gives accurate results for quantities of corticosterone as low as 10 ng/ml.

Results

Table 10 lists the values of the serum chemistries and T_4 for 6- and 12-mo exposures. Individual t-tests did not show any significant effect ($p > 0.05$) between the exposed and sham-exposed groups. Table 11 shows the 6- and 12-mo results of the protein electrophoresis. The gamma globulin showed a marginal effect ($p = 0.0477$, $df = 37$) at 6 mo but not at 12. To compare with the results from the original study, no effect had been observed during the 15 sampling sessions over the 25-mo exposure; therefore, the gamma globulin effect could be due to the variation of the data. The hematology data are shown in Table 12. No significant effect was found in any parameter.

Figure 11 shows the corticosterone level in rats exposed for 6 wk, 6 mo, and 12 mo. The unit is expressed as "micrograms per 100 milliliters" instead of "nanogram per milliliter" so that these values can be more easily compared with those reported in the literature. Statistical evaluation using T-test on these three observations failed to detect any significant difference ($p > 0.05$).

At the first sampling (at 6 wk of exposure) of the original long-term study, the corticosterone level showed a significant difference ($t = 2.06$, $p = .04$, $df = 154$). This effect was not replicated in the present study, so the previously observed effect was most likely a random variation. Inspection of the individual data indicates that data variance was large regardless of the treatment condition--probably related to variations in time from removing an animal from the waveguide cages to the blood sampling. Riley and Spackman (1976) showed that plasma corticosterone increased substantially after 4 min of animal handling. In our study, blood sampling was done within 1 min after the animal was removed from the holding bin. The daily handling procedure--removing the rat from the waveguide cage and weighing and placing it in its holding bin--occurred during 0800-0805 for the group of eight rats to be killed. The necropsy took place during 0830-0850, so the time between the animal's first being touched and the blood sampling was about 30-50 min; however, because of its daily nature the handling stress should have been mild.

TABLE 10. SERUM CHEMISTRY AND THYROXINE DATA OF RATS EXPOSED/SHAM EXPOSED FOR 6 OR 12 MONTHS

Parameter	Exp	Mean	SE	Max	Min
		6 months (N=20)			
Glucose	E	125.5	3.08	153	98
(mg/dl)	S	126.7	2.99	156	104
BUN	E	20.2	0.41	23	16
(mg/dl)	S	20.0	0.39	24	18
Creatinine	E	0.51	0.018	0.6	0.3
(mg/dl)	S	0.53	0.015	0.6	0.4
Sodium	E	144.3	0.35	147	141
(meq/dl)	S	143.9	0.46	146	138
Potassium	E	6.18	0.20	8.9	4.9
(meq/dl)	S	6.11	0.21	8.0	4.8
Chloride	E	102.1	0.27	104	100
(meq/dl)	S	102.2	0.39	106	99
Carbon Dioxide	E	26.9	0.58	32	23
(meq/dl)	S	26.6	0.53	30	20
Uric Acid	E	1.52	0.08	2.2	0.9
(mg/dl)	S	1.64	0.12	3.1	1.0
Total Bilirubin	E	0.05	0.01	0.2	0.0
(mg/dl)	S	0.08	0.01	0.1	0.0
Ionized Calcium	E	4.77	0.11	6.7	4.4
(mg/dl)	S	4.77	0.10	6.5	4.3
Calcium	E	10.1	0.24	14.4	9.3
(mg/dl)	S	9.95	0.19	13.4	9.3
Phosphorus	E	5.36	0.10	6.1	4.4
(mg/dl)	S	5.28	0.11	6.1	4.4
Alkaline Phosphatase	E	159.3	6.17	246	116
(U/l)	S	160.2	3.20	193	131
LDH	E	692.8	58.2	1157	252
(U/l)	S	792.4	61.9	1633	405
SGOT	E	123.6	6.29	177	70
(U/l)	S	132.2	9.53	282	82
SGPT	E	42.7	1.45	60	33
(U/l)	S	39.9	1.53	60	31
Cholesterol	E	106.8	5.70	165	49
(mg/dl)	S	97.9	3.75	137	64
Triglycerides	E	104.1	8.71	220	46
(mg/dl)	S	103.1	7.71	173	55
Total Protein	E	6.21	0.06	6.7	5.7
(g/dl)	S	6.08	0.05	6.6	5.8
Albumin	E	3.43	0.04	3.8	3.1
(g/dl)	S	3.43	0.03	3.6	3.2
Globulin	E	2.78	0.06	3.1	2.4
(g/dl)	S	2.66	0.05	3.2	2.2
Albumin/Globulin	E	1.26	0.03	1.5	1.0
	S	1.31	0.03	1.6	1.1
T4	E	3.48	0.14	4.9	2.4
(µg/dl)	S	3.57	0.14	4.9	2.2

TABLE 10. (continued)

Parameter	Exp	Mean	SE	Max	Min
		12 months (N=20)			
Glucose	E	124.4	3.74	150	85
	S	125.7	3.35	150	100
BUN	E	17.8	0.35	23	16
	S	18.5	0.64	29	16
Creatinine	E	0.64	0.02	0.8	0.5
	S	0.71	0.05	1.2	0.5
Sodium	E	143.7	0.40	148	140
	S	144.8	0.60	150	140
Potassium	E	5.86	0.22	8.0	4.9
	S	5.81	0.18	7.5	4.8
Chloride	E	102.4	0.33	105	98
	S	102.7	0.55	107	98
Carbon Dioxide	E	27.1	0.46	30	23
	S	28.0	0.54	31	22
Uric Acid	E	1.52	0.08	2.5	1.0
	S	1.40	0.07	2.1	1.0
Total Bilirubin	E	0.11	0.02	0.2	0
	S	0.14	0.02	0.3	0
Ionized Calcium	E	4.77	0.04	5.0	4.4
	S	4.78	0.03	4.9	4.4
Calcium	E	10.1	0.07	10.6	9.4
	S	10.1	0.08	10.5	9.0
Phosphorus	E	5.29	0.06	5.7	4.6
	S	5.44	0.07	6.2	4.9
Alkaline Phosphatase	E	168.9	6.54	248	130
	S	166.6	7.63	236	114
LDH	E	723.6	66.7	1148	358
	S	667.0	51.9	1098	332
SGOT	E	138.8	8.80	7	79
	S	129.9	7.69	1	69
SGPT	E	48.2	3.49	89	31
	S	47.0	2.78	74	31
Cholesterol	E	196.7	12.3	314	114
	S	189.2	12.4	314	121
Triglycerides	E	187.6	16.0	328	96
	S	160.9	12.9	290	78
Total Protein	E	6.21	0.05	6.4	5.8
	S	6.18	0.06	6.8	5.8
Albumin	E	3.27	0.04	3.5	2.9
	S	3.27	0.05	3.6	2.7
Globulin	E	2.94	0.04	3.2	2.6
	S	2.92	0.05	3.3	2.4
Albumin/Globulin	E	1.12	0.02	1.3	0.9
	S	1.12	0.03	1.4	0.8
T4	E	4.02	0.24	6.2	2.6
	S	4.01	0.24	6.7	2.2

TABLE 11. PROTEIN ELECTROPHORESIS DATA OF RATS EXPOSED/SHAM-EXPOSED FOR 6 OR 12 MONTHS

	EXP	N	Mean	SE	Max	Min
			6 months			
Albumin	E	20	49.6	2.60	62	4.5
(%)	S	19	51.6	0.66	57	48
Alpha 1	E	20	19.6	1.33	33	2.5
(%)	S	19	21.0	0.69	26	15
Alpha 2	E	20	7.65	0.23	9	5
(%)	S	19	8.26	0.31	11	6
Beta	E	20	15.7	0.25	18	14
(%)	S	19	15.8	0.26	18	14
Gamma	E	20	4.80*	0.65	15	2
(%)	S	19	3.32*	0.29	6	2
			12 months			
Albumin	E	20	55.0	1.49	69	46
	S	20	53.9	1.70	69	35
Alpha 1	E	20	18.3	1.41	27	7
	S	20	19.6	1.72	38	5
Alpha 2	E	20	8.30	0.38	12	5
	S	20	8.40	0.27	11	6
Beta	E	20	6.15	0.46	11	3
	S	20	5.35	0.44	9	2
Gamma	E	20	12.5	0.47	16	9
	S	20	13.0	0.49	17	9

* $p < 0.05$

TABLE 12. HEMATOLOGY DATA OF RATS EXPOSED/SHAM-EXPOSED FOR 6 OR 12 MONTHS

	EXP	N	Mean	SE	Max	Min
			6 months			
WBC	E	20	6.40	0.39	8.8	0.3
	S	20	6.97	0.19	8.7	5.5
RBC	E	20	8.03	0.06	8.7	7.6
	S	20	8.01	0.05	8.4	7.6
HGB	E	20	15.5	0.09	16.4	14.6
	S	20	15.4	0.07	15.8	14.7
HCT	E	20	43.7	0.43	46.9	40.0
	S	20	43.5	0.32	45.7	40.3
MCV	E	20	54.4	0.39	58	51
	S	20	54.3	0.31	57	52
MCH	E	20	19.3	0.10	20.2	18.2
	S	20	18.9	0.39	20.0	11.9
MCHC	E	20	35.5	0.21	36.8	33.8
	S	20	35.4	0.20	37.7	34.4
Lymphocytes	E	20	69.4	4.76	87	6
	S	20	76.0	4.09	92	8
Mono	E	14	3.14	0.54	8	1
	S	15	2.53	0.35	4	1
Eosin	E	17	2.82	0.52	9	1
	S	12	2.58	0.48	6	1
			12 months			
WBC	E	20	4.64	0.30	7.0	2.6
	S	20	4.99	0.18	6.4	3.6
RBC	E	20	7.69	0.08	8.4	7.0
	S	20	7.59	0.09	8.0	6.9
HGB	E	20	15.1	0.11	16.0	14.5
	S	20	15.2	0.14	16.1	13.9
HCT	E	20	44.2	0.87	51.0	37.5
	S	20	43.6	0.90	49.6	35.4
MCV	E	20	57.4	0.72	61.0	51.0
	S	20	57.3	0.74	63.0	51.3
MCH	E	20	19.7	0.15	20.8	18.7
	S	20	20.0	0.15	21.3	18.6
MCHC	E	20	34.5	0.62	39.8	31.4
	S	20	35.0	0.60	39.4	29.6
Neutrophil	E	20	1.31	0.14	2.59	0.38
	S	20	1.53	0.17	3.52	0.69
Lymphocyte	E	20	3.12	0.24	5.24	1.58
	S	20	3.17	0.24	5.16	0.13
Mono	E	20	130.9	38.1	748	0
	S	20	90.0	25.6	495	0
Eosin	E	20	55.3	12.4	162	0
	S	20	44.3	12.5	196	0

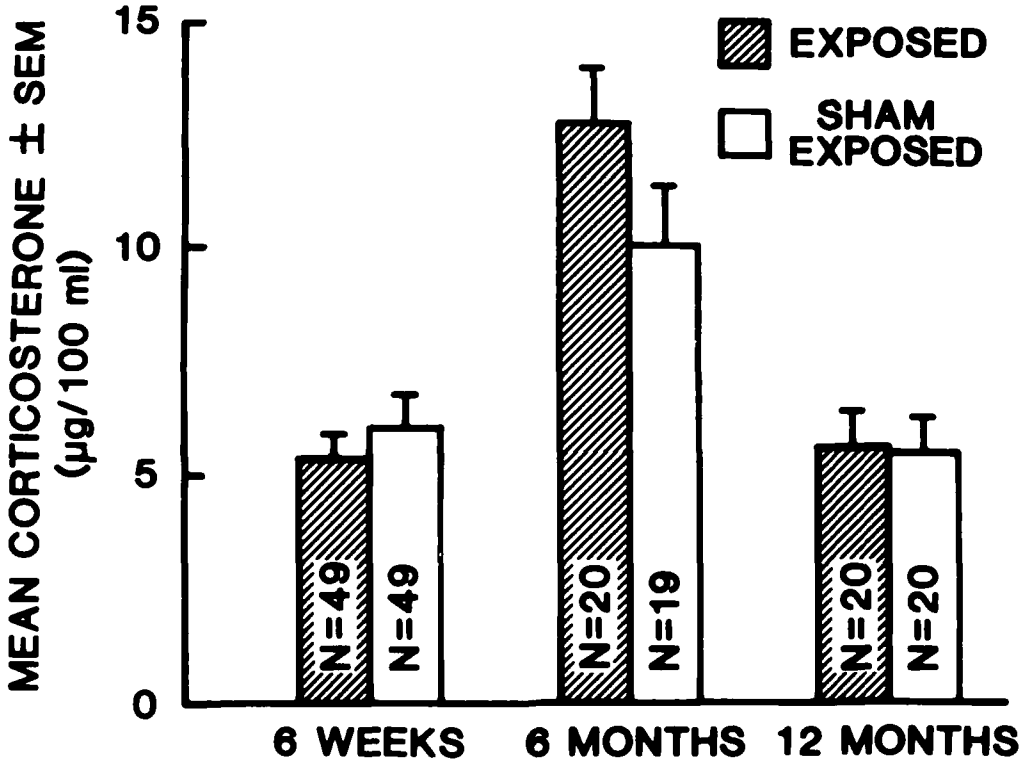

Figure 11. Corticosterone levels in rats exposed for 6 wk, 6 mo, and 12 mo.

Gross Pathological and Histopathological Evaluation

During this experiment all animals were histologically evaluated at death. Documentation of morphological lesions helps to provide a definitive diagnosis of any organ system abnormally present and validates the immune competency studies.

A comparison of spontaneous and age-related lesions in the exposed and sham-exposed animals in this study and in animals of the previous project will help expand the data base on the effects of long-term low-level RFR.

Methods

Animals that died spontaneously were necropsied and the tissues fixed as rapidly as possible. Prior to euthanasia the final-sacrifice animals were examined for any clinical evidence of disease or deviation from normal in physical appearance and behavior; any defects were described and classified at that time. At necropsy, living rats were anesthetized in a halothaneoxygen chamber and euthanized by rapid exsanguination via the carotid or brachial arteries or both. This method minimizes anoxic or agonal hemorrhages and hypostatic congestion. It also allows collecting the maximum volume of blood for serum chemistry and immune system evaluation. If an animal died spontaneously, the clotted heart blood was collected and the serum extracted for possible serology. The necropsies followed a rigid protocol and were done by a veterinary pathologist or a trained research technician under the pathologist's supervision.

Results

Histopathological results for the 6- and 12-mo exposures are listed in Table 13. After the 6-mo exposure, 75 lesions were observed in the exposed group and 62 in the sham-exposed. The 12-mo group had 82 lesions in exposed rats and 75 in controls. Statistical analysis of the total or individual lesions shows no difference between the two groups for both 6- and 12-mo exposure, except peribronchiolar lymphoretical proliferation did increase significantly in 12-mo exposed rats (comparison of binomial proportions, $p = 0.0046$). In general, the occurrence of peribronchiolar

lymphoreticular proliferation was high in both exposed and sham-exposed groups. Six neoplastic lesions (5 benign, 1 malignant) were found in the 12-mo animals. The occurrence was too low to make any statistical comparison meaningful.

TABLE 13. HISTOPATHOLOGICAL RESULTS OF 6- AND 12-MONTH EXPOSURES

Lesion	6 Months Exp	6 Months SE	12 Months Exp	12 Months SE
Adrenal				
adenoma, cortical				1
cellular alteration	7	4	12	8
hyperplasia			2	4
Aorta				
medial calcification			1	
Colon				
subacute nonsuppurative colitis				1
Epididymus				
sperm granuloma		1		
Esophagus				
megaesophagus	1			
Heart				
antoni type a neuronoma			1	1
atrioventricular valves, myxofibromatous degeneration	2	2		
cardiomyopathy	1	1	3	3
cartilaginous foci, subaortic valve	2	1	6	5
fibrosis, tricuspid valve				1
foci of cartilage, base of aortic valve	5	3	1	
myocarditis, multifocal			1	1
Inguinal lymph node				
abscess		1		
Kidney				
chronic glomerulonephropathy	5	4	13	11
chronic interstitial nephritis		3		
cystic tubules	4	5		
Liver				
mononuclear cell infiltration	2			
microfocal granuloma, single			1	
single foci of chronic hepatitis		1		
Lung				
blood inhalation			1	2
peribronchiolar lymphoreticular proliferation	20	20	19	10
petechial hemorrhages		1		
granulomatous inhalation pneumonia	1			
Mandibular sg				
nonsuppurative periductal sialoadenitis	1			
periductal lymphoreticular infiltration		1		

TABLE 13. (continued)

Lesion	6 Months Exp	6 Months SE	12 Months Exp	12 Months SE
Pancreas				
chronic focal pancreatitis				1
ductal lithiasis				2
eyelet cell adenoma				1
Parotid				
nonsuppurative periductal adenitis			1	
periductal lymphoreticular proliferation		1		
Pituitary				
cyst	5	3		
Preputial gland				
abscess	2	1	3	3
cystic pyogranulomatous adenitis			1	3
chronic adenitis	2	1		
cystic hyperplasia	2			1
Prostate				
chronic nonsuppurative prostatitis	1	2		
granulomatous prostatitis	1			
keratin cyst			1	
nonsuppurative adenitis				1
Right testicle				
atrophy, tubular				1
Salivary gland				
periductal adenitis	4	3	2	5
Skin				
pilomatricoma, back, benign				1
Small intestine				
mucoid cystic adenocarcinoma			1	
Stomach				
gastric squamous cell papilloma, diffuse				1
ectopic pancreas in gastric wall	1			
squamous papillomatosis	1			
Testes				
bilateral hypoplasia		1		
dystrophic seminiferous tubular mineralization	1			
Thyroid				
atrophy, diffuse			6	2
hyperplasia, c-cell			1	
follicular squamous metaplasia		1	1	
Trachea				
subacute tracheitis	1			
Zymbals gland				
chronic adenitis	3	1	4	5
Total lesions	75	62	82	75

The pathological results of the original study had indicated that the only effect on nonneoplastic and neoplastic lesions, including both benign and malignant tumors, was a statistically significant increase of the total primary malignant tumors in the exposed animals. Even that occurrence (18 versus 5 in 100 exposed and 100 control animals) was within the normal variation of tumor incidence in chronic studies. The mathematical significant difference is provocative; any biological significance is questionable. After the histopathological examination of the animals in this study, we could not answer the question of tumor incidence because very few tumors developed within the 12 mo of the experiment.

METABOLISM

Two distinct actions of microwave radiation on mammals can, in principle, be distinguished: 1) the hypothesized direct effects on biomolecules and cell structure through as yet undemonstrated mechanisms and 2) the inescapable and demonstrable thermal consequences of the heat produced within biological material during exposure. Although the exposure levels used in the original long-term study were decisively below those known to produce obvious thermal damage to cellular processes, some degree of life prolongation and a reduction in cancer incidence in the exposed population may be a side effect of minor thermal energy accumulated throughout a lifetime of exposure (Sacher, 1977). Such an effect could occur at ambient temperatures below thermoneutrality, depending on how the average male rat metabolizes the thermal energy deposited within it. One option is that it uses the absorbed energy to maintain thermal equilibrium with the ambient environment, as an alternative to using internal chemical stores of energy. This lowered requirement for chemical energy would then result in reduced food intake, oxygen consumption, and total body mass and/or total fat stores and in the paradoxical finding of increased longevity (cf. McCay et al., 1939; Berg and Simms, 1960; Sacher and Duffy, 1978).

No differences between exposed and sham-exposed animals in the long-term study were observed in terms of longevity or in any of the metabolic measurements used, nor for the measures of general health. The data suggest that exposure to microwave radiation at an average lifetime SAR of approximately 0.25 W/kg was so minimal as to be of little metabolic consequence with respect to the total energy balance of the exposed animal population.

Objectives

The above suggestion of minimal impact of a lifetime exposure to microwave radiation lacks the support that would be provided by a set of positive control experiments that demonstrated the reliability and

sensitivity of measurement techniques used. The statement made by the original study will have meaning only within the context of the dose-response relationship of specific endpoints at various microwave exposure levels.

The objective of this phase of the research is to provide an understanding of the dose-response relationships of the health profile and metabolic and immunological parameters assessed in the original study. Additional emphasis will be placed on the metabolic relationships through factorial combination of multiple microwave-exposure levels and ambient temperatures.

Scope of Work

To determine a dose-response relationship for demonstrable microwave effects, we exposed nine groups of 10 rats (90-day-old males, same supplier as before) each to 2450-MHz CW microwave radiation for 6 wk. The nine treatment conditions represent a factorial combination of three microwave power densities and three ambient temperatures. The power densities (5, 10, and 15 mW/cm^2) all exceeded the level used in the original study by at least a factor of 10. The corresponding average SARs were 2.5, 5, and 7.5 W/kg. Ten animals were sham exposed and served as controls at each ambient temperature (17.8, 22.2, and 26.7°C [64, 72, 80°F]). The relative humidity was maintained at 55 $\pm$ 10%.

One of the two SPF animal-exposure rooms was used for this study. The logistics of the exposure facility are such that three exposure levels can be maintained simultaneously in three separate alcoves, with the ambient temperature held constant at one selected level. Therefore, only three actual exposure periods had to be conducted, one at each of the three ambient temperatures. Each alcove contained 10 animals housed in the exposure apparatus, and 10 sham-exposed animals were partitioned between the three alcoves to serve as control subjects for each ambient temperature.

During each exposure period, metabolism data were collected daily from the rats housed in each alcove, including assessment of body weight and food and water consumption. Respiratory gas exchange (i.e., oxygen consumption and carbon dioxide production) was measured simultaneously on four animals in airtight metabolism chambers. Three of these animals were microwave exposed, each at a different power density, and the fourth was sham-exposed. Each animal was in the metabolism chamber for 2 days. During the 6-wk exposure, the metabolic gas exchange was measured twice for all rats.

At death, serum chemistry, protein electrophoresis, T_4, and corticosterone were measured. Also, gross pathological and histopathological examinations were made after sacrifice of animals. As part of the immunological competence studies described before, the spleen, thymus, and bone marrow tissues were removed for testing.

Assessment Rationale

To survive, all mammalian species must maintain internal body temperature within very narrow limits. Much literature details the autonomic mechanisms of thermal regulation as well as the behavioral strategies evolved by each species to cope with their ever-changing thermal environment.

Deposition of thermalizing radiant energy is an inescapable consequence of microwave exposure, particularly at higher power densities. At times bioeffects research literature inadvertently focuses attention on the obvious cellular damage accompanying elevated tissue temperatures, furthering misunderstanding of the less dramatic responses of the irradiated organism. Although an elevated rectal temperature indicates that various regulatory mechanisms have failed to maintain normal thermal equilibrium in response to an externally imposed thermal burden, the finding of a normal rectal temperature (i.e., thermal regulatory mechanisms

have not failed) is often cited as if to indicate that only a "non-thermalizing" level of microwave exposure had been used.

Recently a few reports have appeared describing certain compensatory adjustments effected during microwave exposure, such as reduction in food intake (Gage, 1979; Chou et al., 1983a), reduction in oxygen consumption (Phillips, 1975; Ho and Edwards, 1977), and increased evaporative heat loss (Gordon, 1982). Some researchers have begun to characterize behavioral thermoregulatory strategies used by various species during exposure to microwave radiation, including the lizard (D'Andrea et al., 1978), monkey (deLorge, 1976; Adair and Adams, 1980), and rat (Stern et al., 1979).

Although the behavioral and physiological systems involved in thermoregulation in the various ambient environments are understood in general, no systematic investigation has extended this understanding to the adaptive processes associated with long-term exposure to microwave radiation. The ubiquitous argument concerning thermal versus nonthermal effects demands clarification of the physiological and behavioral strategies mounted in response to microwave energy absorption. Research to clarify these strategies will help us understand how complex thermoregulatory adaptations can affect other physiological systems, thereby producing so-called nonthermal microwave effects.

Ambient temperature is the most effective determinant of thermoregulatory adaptation in animals. Therefore, understanding the thermoregulatory-system response to the interaction of microwave energy deposition at various ambient temperatures is necessary. Thermoneutrality is defined as the ambient temperature range at which the minimum level of metabolic activity is observed, or the TNZ. For the rat this level has been assessed as 28-33^{o}C (Herrington, 1940). At temperatures below the TNZ, metabolism rises due to increased heat production associated with decreasing temperature. At temperatures above the TNZ, metabolism rises due to increased motor activity associated with behavioral thermoregulation. This temperature range of TNZ has been assumed to approximate an index of comfort (Folk, 1966; McNab, 1970; Porter and Gates, 1969).

The classical definition of thermoneutrality has been relaxed somewhat in recent years to include a wider range of behavioral thermoregulatory responses (Bianca, 1974). The usual practice of breeding and maintaining rats within the TNZ has been questioned, and suggestions have been made that a lower ambient temperature (20-25^{o}C) may provide a less stressful and more healthy environment for the laboratory rat (Weihe, 1965; Poole and Stephenson, 1977).

Food intake of the rat varies as a function of ambient temperature; highest intake accompanies ambient temperatures near 0^{o}C and decreases linearly to a minimum at 35-38^{o}C (Brobeck, 1948; Hamilton, 1963; Jakubczak, 1976). Previous studies in this laboratory that varied ambient temperature and monitored food intake in rats exposed to various microwave power densities have found that food intake decreased as an additive function of both rising ambient temperature (21-26^{o}C) and increasing SAR (0.8-7.2 W/kg) (Moe et al., 1976; Lovely et al., 1977).

As ambient temperature rises (but below the TNZ), the rat uses additional physiological and behavioral strategies to thermoregulate. These include dramatic reductions in activity (Hainsworth et al., 1968), initiation of saliva spreading (Hainsworth, 1968), and vasodilation of the tail (Rand et al., 1965; Hellstrom, 1975; Dawson and Keber, 1979).

One outcome of these thermoregulatory adjustments is an increase in the level of stress associated with the environment, as measured by circulating levels of plasma corticosterone. Some investigators have demonstrated that individual rats have varying capacities to deal with thermal stress and implement different strategies to cope with experimentally induced thermal burdens (Robinson et al., 1968; Blackmore, 1970; Hubbard et al., 1976; Isobe et al., 1980). As mentioned in the immunology section, the stress associated with thermoregulatory adjustments may be a determining factor in the alteration of immunological responses. Therefore, plasma corticosterone was assayed on each animal just prior to sacrifice, as an indicator of the perceived demands placed on it by the environment. This information will serve as part of the dose-response data base and help validate the immunological assessments.

Animals from each of the nine experimental conditions were sacrificed and necropsied at the termination of each study. The spleen, thymus, and

bone marrow tissues were removed and analyzed and the data used to form a dose-response analysis of the altered immunological response.

Serum chemistry, hematological, protein-electrophoresis, and T_4 determinations, as well as gross pathological and histological examinations, provide a measure of the general health of each animal prior to sacrifice, for supportive evaluation of the immunological data. Moreover, all these endpoints form part of a larger dose-response evaluation of the endpoints used in the original study.

Methods

Body weight and food and water consumption of each animal were measured daily throughout the course of each study. The method most widely used to determine metabolic rate involves measuring respiratory gas exchange. The use of this measurement as an index of metabolic activity is based on the principle that oxygen is consumed and carbon dioxide is produced in direct proportion to the intensity of metabolic activity. The sum of all metabolic activity within an organism can be measured at the rate at which oxygen is removed from the air in the immediate environment of the organism.

Many technical problems are associated with measuring gas exchange as an index of metabolism. For example, the oxygen analyzers commercially available actually measure the partial pressure of oxygen in the sampled air; therefore the temperature of the air, its relative humidity, the barometric pressure, and the chamber airflow rates all affect the calculation of oxygen consumption. To simplify the gas exchange monitoring system so that it could be routinely performed as part of a standard daily procedure, we chose the equipment for this project to minimize the need to precisely monitor these additional endpoints.

The oxygen analyzer used is a dual-channel unit that has two sample cells for measuring the partial pressure of oxygen in two airstreams simultaneously, expressed as percent oxygen (Applied Electrochemistry, Model S-3A, Sunnyvale, CA). Both cells are located in the same housing

and share a common internal reference to the ambient oxygen concentration, temperature, and barometric pressure. The output of each cell can be displayed on a front panel meter or recorded by remote equipment with a resolution of 0.01%. When the unit is operated in the different mode, the difference between the outputs of the two cells can be obtained with a resolution of 0.001%.

To take advantage of the higher resolution and to further isolate the measurements from the effects of fluctuating ambient conditions, we operated the analyzer in the differential mode. The first cell was always sampling a common source of ambient air entering the metabolism cages, and the second cell alternately sampled air exhausted from one of the four metabolism cages. The critical measurement for calculating oxygen consumption is the oxygen-concentration difference between the incoming and the exhausted air, and the analyzer provides this measurement continuously, independently of changes in ambient conditions. This mode of operation also eliminated the need for daily instrument calibration with a gas of known absolute oxygen concentration. Since both cells were referenced internally to ambient conditions and cell #1 was continuously measuring ambience, the daily calibration procedure simply adjusts the instrument to indicate the accepted ambient oxygen concentration of 20.90%. This value applies only to air samples from which the vapor pressure of water has been eliminated through drying of the air sample. Prior to gas sampling, therefore, surge bottles filled with anhydrous calcium sulfate, $CaSO_4$ (Drierite), were connected in-line with the airstream exiting from the metabolism cages.

The accuracy of the oxygen measurements was little affected by small perturbations in the airflow rates through the system. It is very critical, however, that the daily calibration procedure be performed under airflow conditions identical to those when actual gas exchange measurements are taken. The airflow rates chosen for this system were in the range of highest stability for each particular measuring device used. The rate at which air must be drawn through each chamber to sustain a rat has been experimentally determined to be approximately 2300 ml/min at 21°C. This

value was evaluated at various ambient temperatures to ensure its acceptability prior to final experimentation. The airflow through each of the four chambers was continually maintained by a master pump adjusted to draw approximately 10 L/min. This flow was not monitored directly, only as the sum of the four airflows through the chambers. The balance of airflow between the chambers was controlled by needle valves. The mass flow through each chamber was electronically monitored by flow meters (Kurz Instruments, Model #565) inserted in the air lines leaving the chambers. Temperature sensors were placed in the intake and exhaust air lines of each chamber so that chamber temperature rise would be indicated.

The air flowing into each chamber was derived from a common inlet, and sampling for cell #1 was made at this point. A PDP 11/23 microcomputer-based monitoring unit electrically switched four solenoid-actuated on/off flow valves so that the air sampled for cell #2 was alternately drawn from each of the four chambers at 30-s intervals. The same line drawing air to cell #2 for oxygen concentration measurements also passed through the sensor for the carbon dioxide analyzer. This analyzer (Applied Electrochemistry, Model CC-2) is the companion instrument to the oxygen analyzer with a 0.01% resolution. The microcomputer logged all data pertaining to each unit, including airflow rate, oxygen and carbon dioxide concentration, and air temperatures. These data were collected and retained by the microcomputer for up to 23 h daily and then off-loaded to the PDP 11/34 computer for storage.

Colonic and tail temperatures of rats as well as inlet and outlet temperatures of the metabolic chamber were measured with thermistors. All other measurements on body mass, food/water consumption, health profile, immunological assessments, and histopathological evaluations were the same as in the 6- and 12-mo study described earlier in this report.

Results

Mortality

Table 14 lists the number of animal deaths during the three 6-wk exposures. At 17.8°C and 15 mW/cm^2, two rats died--one during the 3d week and another during the last week. At 22.2°C and 15 mW/cm^2, two rats died during the 1st week (day 4); these were replaced. At 26.7°C and 15 mW/cm^2 all 10 rats died the 1st day. These were replaced but at 7.5 mW/cm^2; two of these died during the 1st week. At 26.7°C and 10 mW/cm^2, 7 of the 10 rats died on the 1st day; two of these seven were replaced, but all five remaining rats died within 3 days. None of the animals in the 0 and 5-mW/cm^2 groups died in any of the temperature studies.

TABLE 14. EFFECTS OF MICROWAVE EXPOSURE AND TEMPERATURE ON RAT MORTALITY IN CIRCULAR WAVEGUIDES

	Animal Death				
	Power density (mW/cm^2)				
T_I (°C)	0	5	7.5	10	15
17.8	0	0	NA	0	2
22.2	0	0	NA	0	2
26.7	0	0	2	10	10

The differences in temperature at the outlet and inlet of the chamber over 1 day are presented in Figures 12-14. At 17.8°C the 5-mW/cm^2 exposure group was lower than the others. The same result was seen at 22.2°C; the drop seen in the 15-mW/cm^2 group at 0600 was due to the death of an animal. At 26.7°C the exposed rats died between 1800 and 2000 in both the 10- and 15-mW/cm^2 groups, which caused the drop in temperature. These data indicated that live-animal response plays an important part in the rise of chamber temperature.

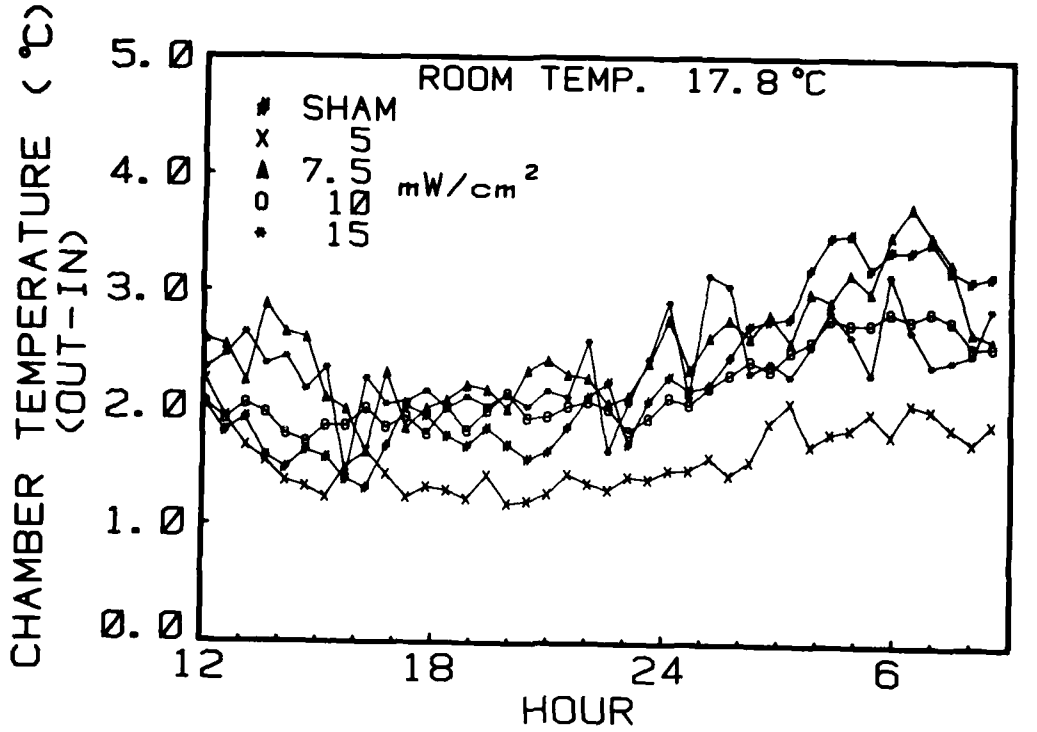

Figure 12. Increase in chamber temperature at 17.8°C.

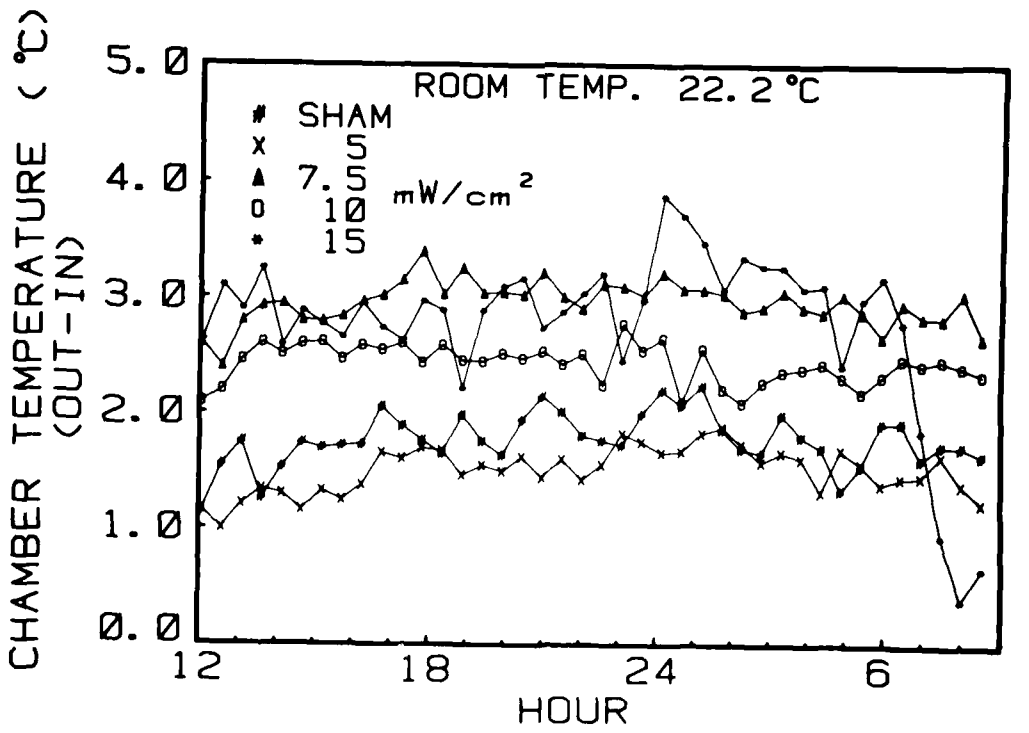

Figure 13. Increase in chamber temperature at 22.2°C.

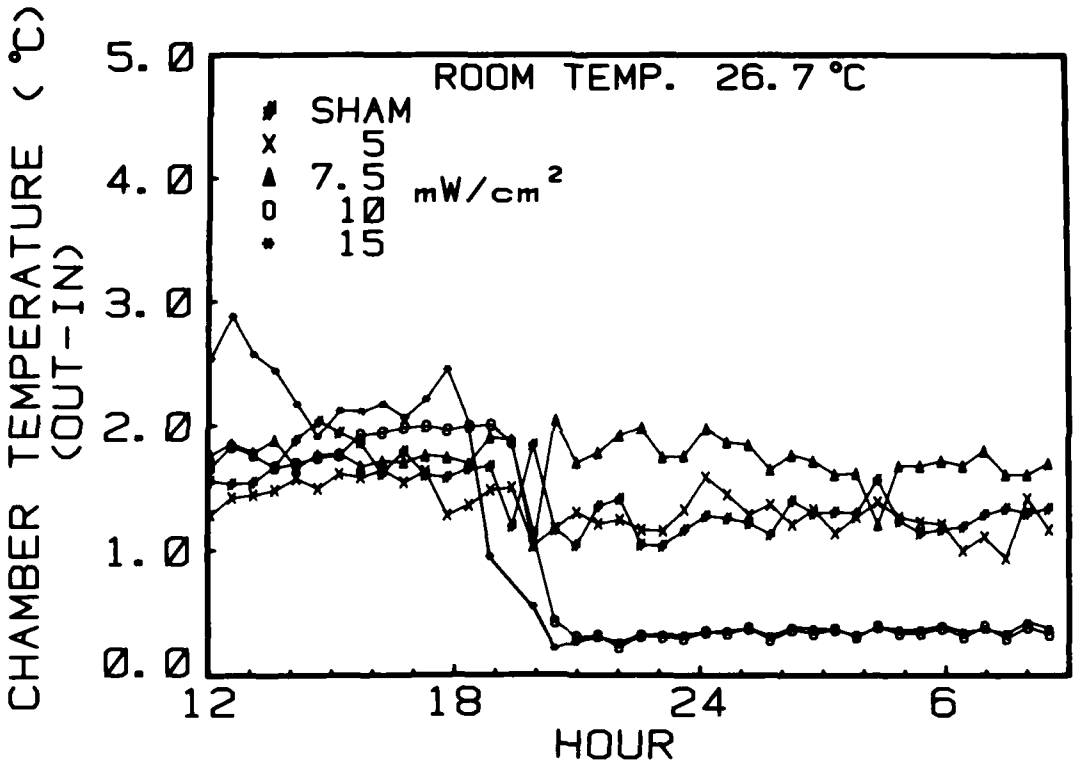

Figure 14. Increase in chamber temperature at 26.7°C.

Body Temperature

Colonic and tail temperatures were measured on a separate group of rats. The tail temperature had large variations and did not provide any meaningful results for a small sample size; therefore, only colonic temperature measurements were completed. (See Table 15.) Each set of data was an average of 2-5 measurements on at least two rats and at 5-h and 1-, 2-, and 3-day exposures. At 0- and 5-mW/cm^2 exposures, there were no significant body temperature changes for any ambient temperature. Changes larger than 1^{o}C occurred at 15 mW/cm^2 at all three ambient temperatures and at 7.5 and 10 mW/cm^2 at 26.7^{o}C. These body temperature rises correlate with the animal deaths (Table 14). The data indicate that the rats survived the 6-wk experiment if their body temperature remained regulated less than 1^{o}C above their normal temperature. Above this limit, the animals' thermoregulation failed and they eventually died. Recently Berman et al. (1985) studied the lethality in mice and rats exposed to microwaves as a function of exposure duration and ambient temperatures.

TABLE 15. EFFECTS OF MICROWAVE EXPOSURE AND TEMPERATURE ON RAT COLONIC TEMPERATURE IN CIRCULAR WAVEGUIDES

	Change in Core Temperature (^{o}C)				
	Power density (mW/cm^2)				
T_I (^{o}C)	0	5	7.5	10	15
17.8	0.08±0.17	-0.28±0.19	0.20±0.04	0.00±0.51	1.16±0.59
22.2	0.26±0.13	-0.40±0.30	0.50±0.15	1.00±0.40	6.20±0.20
26.7	0.14±0.25	0.60±0.41	2.52±1.03	3.73±1.53	6.33±1.44

Body Mass and Consumption of Food and Water

Figure 15 presents daily average body mass for the 22.2°C ambient temperature. To simplify statistical analysis, data were averaged weekly and compared using analysis of variance with repeated measures. Figures 16-18 show the weekly averages for 17.8, 22.2, and 26.7°C. Table 16 lists the weekly means and standard errors of body mass. At 17.8°C and 0, 5, and 10 mW/cm^2, growth rates were similar; at 15 mW/cm^2, growth rate apparently decreased. At 22.2°C, growth rate decreased with increasing exposure level. At 26.7°C and 7.5 mW/cm^2, no growth was seen. Statistical analysis using analysis of variance with repeated measures on three sets of data were performed: 1) temperature at 17.8 and 22.2°C and radiation of 0, 5, 10, and 15 mW/cm^2; 2) temperature at 17.8, 22.2, and 26.7°C and radiation of 0 and 5 mW/cm^2; 3) temperature at 26.7°C and radiation of 0, 5, and 7.5 mW/cm^2.

Statistical results for set 1 show significant effects of temperature ($f = 27.75$, $df = 1,72$, $p < 0.0001$); radiation ($f = 18.57$, $df = 3,72$, $p < 0.0001$); time ($f = 176.97$, $df = 5,360$, $p < 0.0001$); time-radiation interaction ($f = 5.96$, $df = 15,360$, $p < 0.001$); and time-temperature-radiation interaction ($f = 1.98$, $df = 15,360$, $p = 0.016$). Detailed analysis pf weekly variations is shown in Table 17. The L, M, and H represent the 17.8, 22.2, and 26.7°C; and the numbers are the power densities. Results for set 2 indicate significant effects of temperature ($f = 6.97$, $df = 2,54$, $p = 0.002$); radiation ($f = 8.71$, $df = 1,54$, $p = 0.0047$); temperature-radiation ($f = 5.18$, $df = 2,54$, $p = 0.0088$); and time ($f = 268.56$, $df = 5,270$, $p < 0.0001$). When combined across time, conditions within the following three groups show no differences: H-5, L-0, L-5; L-0, L-5, M-5, H-0; L-5, M-5, H-0, M-0. Among the groups, there were significant differences at $p < 0.01$.

The 3d set of data showed a significant effect of radiation ($f = 15.09$, $df = 2,25$, $p = 0.0001$); time ($f = 47.89$, $df = 5,125$, $p < 0.0001$); and time-radiation interaction ($f = 14.45$, $df = 10,125$, $p < 0.0001$). An analysis of weekly variations is shown in Table 18.

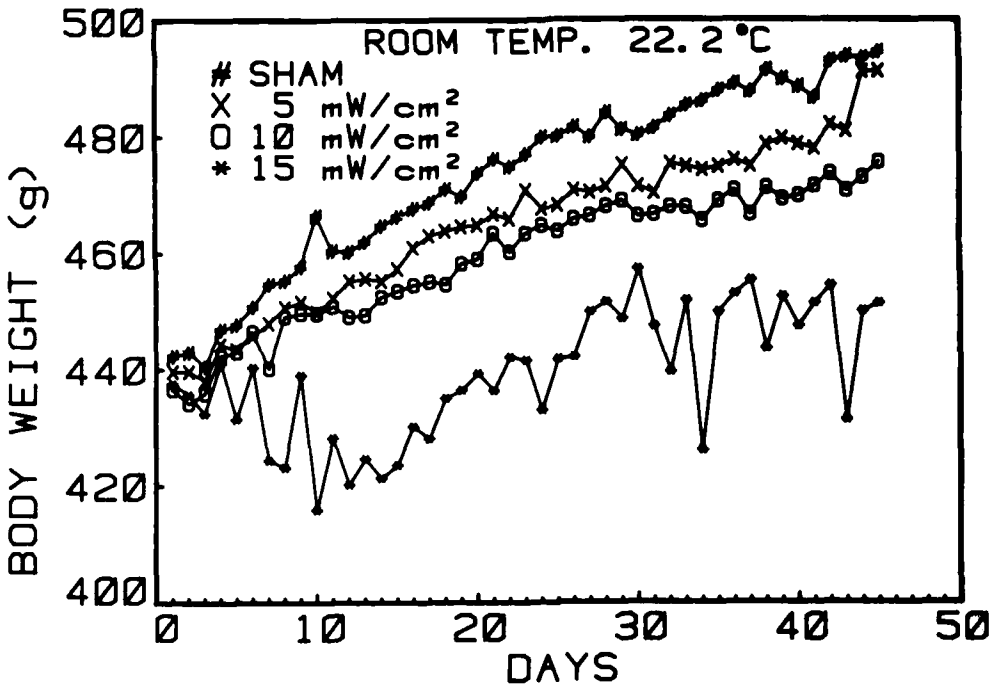

Figure 15. Example of daily average body mass data at 22.2°C.

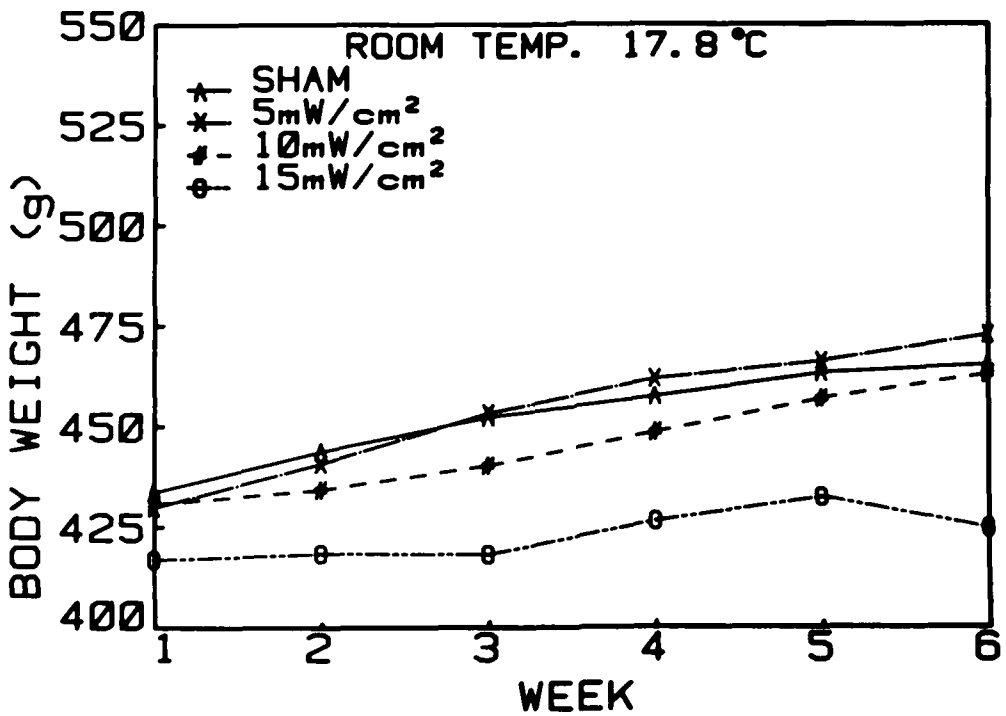

Figure 16. Weekly average body mass at 17.8°C.

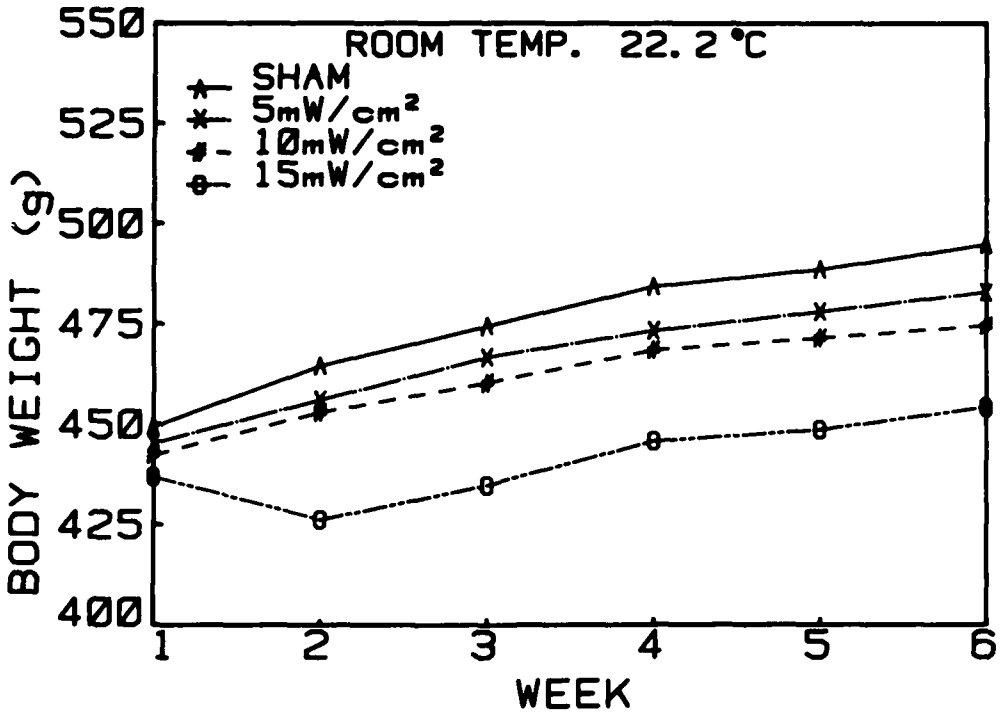

Figure 17. Weekly average body mass at 22.2°C.

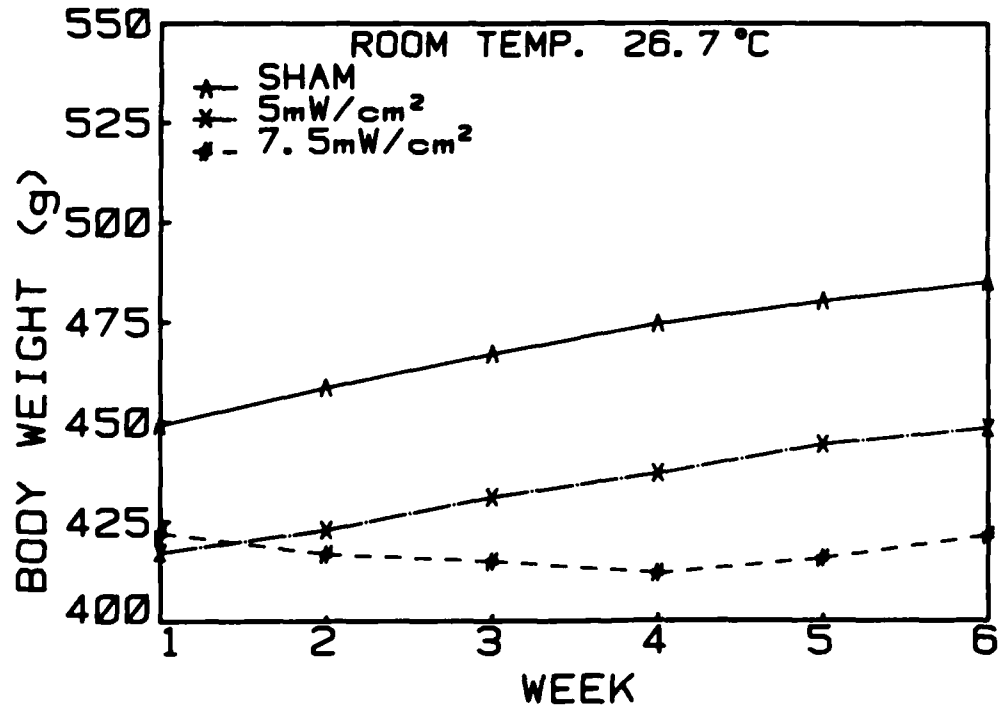

Figure 18. Weekly average body mass at 26.7°C.

TABLE 16. WEEKLY MEANS AND STANDARD ERRORS OF BODY MASS

Weeks	Power Density (mW/cm^2)							
	0		5		10		15	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
T=17.8°C								
1	433.6	4.49	429.8	4.11	430.7	4.74	416.8	7.12
2	443.7	4.81	440.7	4.52	434.2	5.82	418.3	5.28
3	452.2	5.69	453.2	5.34	440.2	6.48	418.1	5.22
4	457.3	6.29	461.7	5.57	448.4	5.15	426.4	3.40
5	463.1	7.72	466.0	6.80	456.7	5.44	432.2	3.45
6	465.2	8.98	472.6	7.21	462.8	5.53	424.6	3.95
T=22.2°C								
1	449.3	3.13	445.2	4.62	442.3	3.00	436.8	2.56
2	464.5	3.73	456.0	5.25	452.9	2.28	426.1	4.02
3	474.4	4.49	466.6	5.50	460.2	2.15	434.7	4.27
4	484.3	5.38	473.3	5.91	468.4	2.56	445.7	5.00
5	488.5	5.79	478.0	6.70	471.4	4.17	448.5	6.04
6	494.7	5.50	482.8	7.08	474.5	6.04	454.1	6.29
T=26.7°C					7.5			
1	449.2	8.38	417.2	5.79	422.0	6.32		
2	458.6	8.47	422.9	5.38	416.7	6.07		
3	467.0	7.75	431.1	5.79	414.9	5.38		
4	474.6	7.37	437.0	5.25	411.9	4.62		
5	480.3	8.16	444.3	6.83	415.6	6.01		
6	485.0	7.15	448.3	8.44	421.4	6.64		

TABLE 17. COMPARISON OF BODY MASS AT DIFFERENT TIMES OF EXPOSURE TO 0, 5, 10, AND 15 mW/cm^2 POWER AT 17.8°C (L) and 22.2°C (M)

Week	Condition							
1	L-15	L-5	L-10	L-0	M-15	M-10	M-5	M-0
2	L-15	M-15	L-10	L-5	L-0	M-10	M-5	M-0
3	L-15	M-15	L-10	L-0	L-5	M-10	M-5	M-0
4	L-15	M-15	L-10	L-0	L-5	M-10	M-5	M-0
5	L-15	M-15	L-10	L-0	L-5	M-10	M-5	M-0
6	L-15	M-15	L-10	L-0	L-5	M-10	M-5	M-0

Body mass increasing from left to right.
Body mass did not differ significantly between underscored conditions.
Between conditions underscored by the same line, body-mass difference is statistically significant at $p < 0.01$.

TABLE 18. COMPARISON OF BODY MASS AT DIFFERENT TIMES OF EXPOSURE TO 0, 5, AND 7.5 mW/cm^2 AT 26.7°C

Week	Exposure level		
1	5	7.5	0
2	7.5	5	0
3	7.5	5	0
4	7.5	5	0
5	7.5	5	0
6	7.5	5	0

Food consumption was analyzed in the same way as body mass. Figures 19-21 show the weekly average food consumption data. The means and standard errors are shown in Table 19. Food consumption apparently drops as power density increases, for all temperatures. Set 1 data (17.8 and 22.2^{o}C; 0, 5, 10, and 15 mW/cm^{2}) showed significant effects of radiation ($f = 103.8$, $df = 3,72$, $p < 0.0001$); time ($f = 5.46$, $df = 5,360$, $p = < 0.0001$); and time-temperature ($f = 7.62$, $df = 5,360$, $p < 0.0001$). When averaged across 6 wk and the two temperatures, differences among the four exposure levels are all significantly different at $p < 0.01$. When averaged across the four exposure levels, differences of temperature effect were significant during first 2 wk only ($p < 0.01$).

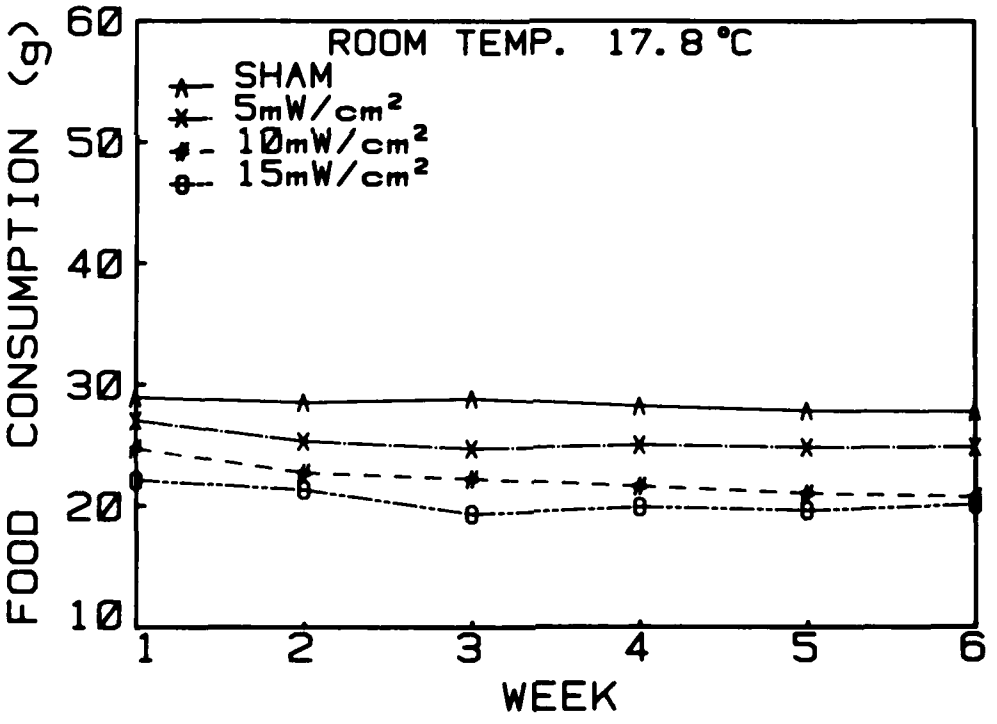

Figure 19. Weekly average food consumption at 17.8^{o}C.

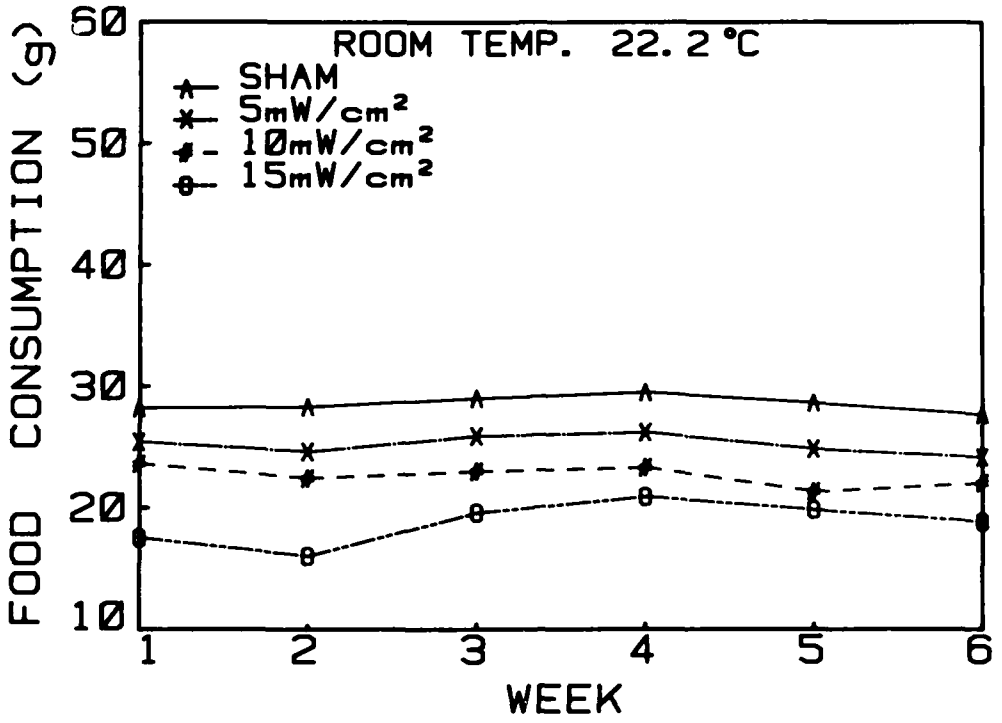

Figure 20. Weekly average food consumption at 22.2°C.

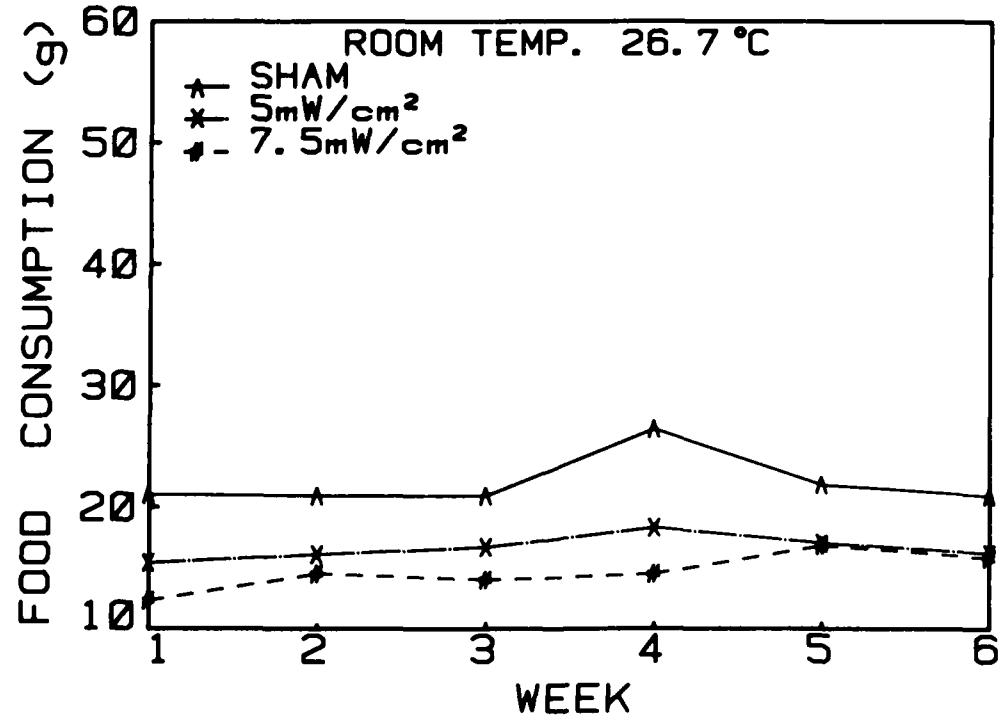

Figure 21. Weekly average food consumption at 26.7°C.

TABLE 19. WEEKLY MEANS AND STANDARD ERRORS OF FOOD CONSUMPTION

	Power Density (mW/cm^2)							
	0		5		10		15	
Week	Mean	SE	Mean	SE	Mean	SE	Mean	SE
$T=17.8^oC$								
1	28.9	0.72	27.0	0.72	24.7	0.76	22.1	1.11
2	28.5	0.41	25.3	0.79	22.7	0.82	21.3	1.17
3	28.8	1.01	24.8	0.98	22.3	0.63	19.3	0.51
4	28.2	1.14	25.0	0.98	21.6	0.57	19.9	0.28
5	27.8	0.73	24.8	0.85	21.0	0.32	19.6	0.51
6	27.7	0.70	24.8	0.76	20.7	0.54	20.1	0.63
$T=22.2^oC$								
1	28.2	1.08	25.4	0.51	23.6	0.38	17.5	1.11
2	28.3	0.70	24.6	0.57	22.4	0.63	16.0	0.95
3	29.0	0.54	25.9	0.73	23.0	1.11	19.6	0.70
4	29.5	0.54	26.2	0.89	23.3	0.95	20.9	0.66
5	28.6	0.76	24.8	0.70	21.3	1.17	19.8	0.70
6	27.6	0.76	24.2	0.73	22.0	1.33	18.8	0.98
$T=26.7^oC$					7.5			
1	21.0	0.73	15.4	0.74	12.3	0.28		
2	20.9	0.47	16.1	0.51	14.5	0.63		
3	20.9	0.73	16.7	0.47	14.0	0.22		
4	26.4	1.52	18.3	0.70	14.5	0.79		
5	21.8	0.66	17.0	0.76	16.8	0.35		
6	20.9	0.47	16.2	0.47	15.8	0.54		

Set 2 analysis (17.8, 22.2, and 26.7°C; 0 and 5 mW/cm^2) indicated that significant effects were of temperature ($f = 121.27$, $df = 2,54$, $p < 0.0001$), radiation ($f = 74.35$, $df = 1,54$, $p < 0.0001$), time ($f = 8.23$, $df = 5,270$, $p < 0.0001$), and time-temperature ($f = 5.29$, $df = 10,270$, $p < 0.0001$). When averaged across 6 wk and the three temperatures, food consumption was significantly reduced for the 5-mW/cm^2 exposed rats when compared to the sham exposed ($p < 0.01$). When the 0 and 5-mW/cm^2 data were combined, differences were seen between the 26.7 and the 17.8/22.2°C groups. No difference was found between the 17.8 and 22.2°C groups.

Results for the set 3 data (26.7°C; 0, 5, and 7.5 mW/cm^2) showed effects of radiation ($f = 55.93$, $df = 2,25$, $p < 0.0001$), time ($f = 13.67$, $df = 5,125$, $p < 0.0001$), and time-radiation ($f = 5.42$, $df = 10,125$, $p < 0.0001$). Analysis across time is shown in Table 20.

TABLE 20. COMPARISON OF FOOD CONSUMPTION AT DIFFERENT TIMES OF EXPOSURE TO 0, 5, and 7.5 mW/cm^2 AT 26.7°C

Week	Exposure Condition		
1	7.5	5	0
2	7.5	5	0
3	7.5	5	0
4	7.5	5	0
5	7.5	5	0
6	7.5	5	0

Daily water consumption showed large variations. Even the weekly averages (Figures 22-24) did not have consistent patterns. The same statistical analysis as for body mass was done on these data. Table 21 shows the weekly means and standard errors of the water consumption. Set 1 data (17.8 and 22.2^{o}C; 0, 5, 10, and 15 mW/cm^{2}) showed effects of radiation ($f = 3.45$, $df = 3,72$, $p = 0.02$); time ($f = 11.4$, $df = 5,360$, $p < 0.0001$); time-temperature ($f = 9.10$, $df = 5,360$, $p < 0.0001$); and time-radiation ($f = 2.31$, $df = 15,360$, $p = 0.0038$). Averaged across the four exposure levels, temperature effect was significant only during week 5.

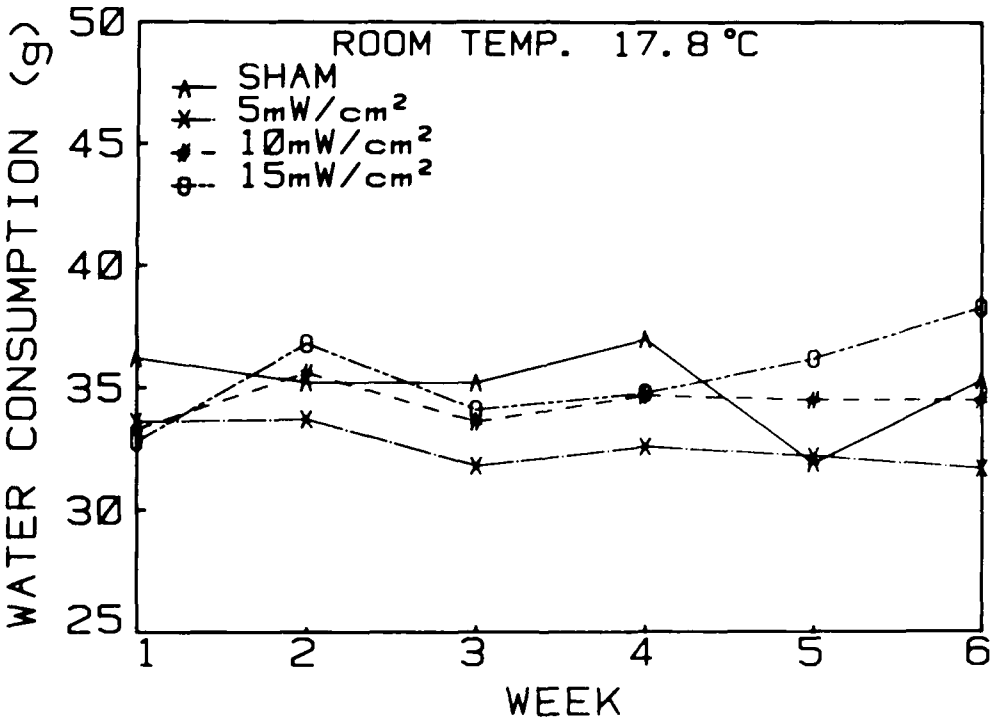

Figure 22. Weekly average water consumption at 17.8^{o}C.

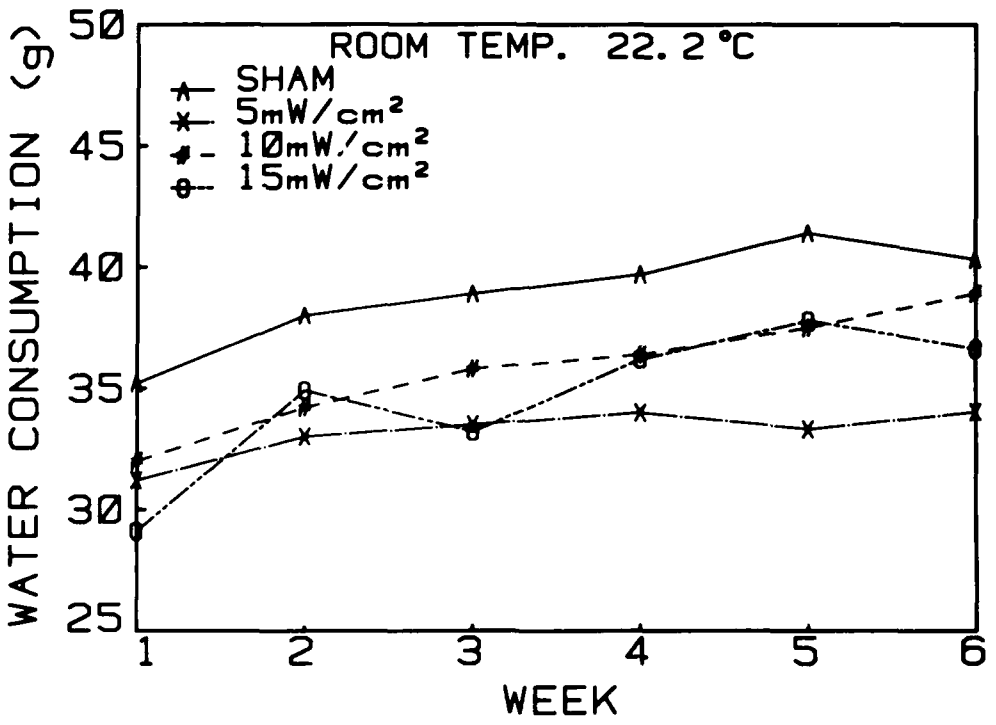

Figure 23. Weekly average water consumption at 22.2°C.

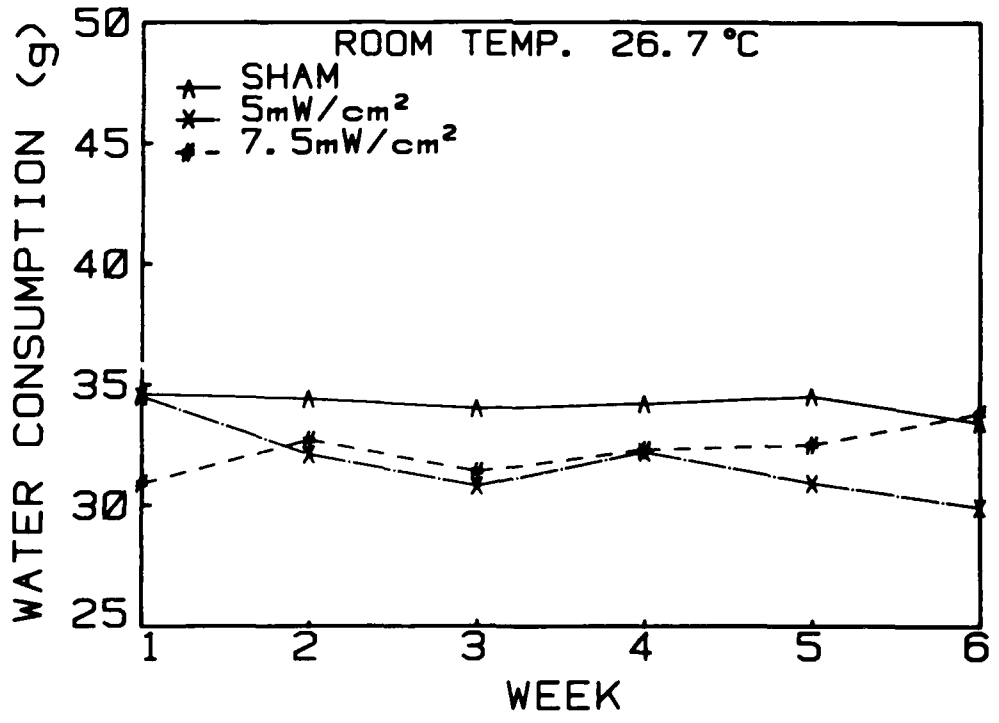

Figure 24. Weekly average water consumption at 26.7°C.

TABLE 21. WEEKLY MEANS AND STANDARD ERRORS OF WATER CONSUMPTION

	Power density (mW/cm^2)							
	0		5		10		15	
Week	Mean	SE	Mean	SE	Mean	SE	Mean	SE
$T=17.8^oC$								
1	36.2	1.11	33.6	0.79	33.3	1.55	32.8	0.76
2	35.2	0.47	33.7	0.57	35.6	1.01	36.8	0.63
3	35.2	0.92	31.8	0.85	33.6	1.64	34.1	1.17
4	37.1	1.83	32.6	0.92	34.7	1.64	34.8	1.17
5	32.0	3.42	32.3	1.20	34.5	1.64	36.2	1.87
6	35.3	1.36	31.8	0.76	34.5	1.48	38.3	1.80
$T=22.2^oC$								
1	35.2	1.99	31.2	1.13	32.0	0.79	29.2	1.83
2	38.0	2.47	33.1	1.11	34.2	0.82	34.9	2.75
3	38.9	2.21	33.5	1.14	35.8	1.52	33.2	1.42
4	39.7	2.25	34.0	1.11	36.5	2.06	36.2	0.82
5	41.4	2.12	33.3	0.98	37.6	1.96	37.8	0.85
6	40.3	2.12	34.0	1.39	38.9	2.44	36.6	1.11
$T=26.7^oC$					7.5			
1	34.6	1.42	34.5	1.64	30.9	1.04		
2	34.4	1.45	32.1	1.61	32.7	0.92		
3	34.0	1.45	30.8	1.01	31.4	1.07		
4	34.2	1.61	32.2	1.23	32.3	1.11		
5	34.5	1.58	30.9	1.26	32.5	1.36		
6	33.4	1.61	29.9	1.64	33.8	1.99		

Set 2 data (17.8, 22.2, and 26.7°C; 0 and 5 mW/cm^2) also showed effects of radiation ($f = 12.35$, $df = 1,54$, $p = 0.0009$); time-temperature ($f = 6.67$, $df = 10,270$, $p < 0.0001$); and time-radiation ($f = 3.07$, $df = 5,270$, $p = 0.0232$). The temperature effect was analyzed weekly for data averaged across two exposure levels. Table 22 shows the results. In general, rats drank more water at 22.2°C than at 17.8°C and less than at 26.7°C, but the significant difference ($p < 0.01$) occurs only at the 5th and 6th wk of exposure. At 26.7°C, the only significant difference was time-radiation ($f = 2.50$, $df = 10,125$, $p = 0.0009$). When analyzed across time, the time-radiation interaction was apparently due to changes of each exposure level across time rather than differences among exposure levels at each time.

TABLE 22. COMPARISON OF WATER CONSUMPTION AT DIFFERENT TIMES OF EXPOSURE TO 0 AND 5 mW/cm^2 AVERAGED AT 17.8°C (L), 22.2°C (M), and 26.7°C (H)

Week	Temperature		
1	M	H	L
2	H	L	M
3	H	L	M
4	H	L	M
5	H	L	M
6	H	L	M

Animal Health Profile

Tables 23-25 tabulate the means and standard errors of the serum chemistry, T_4, protein electrophoresis, and hematology data of the 120 rats exposed to microwave radiation at 0, 5, 7.5, 15 mW/cm^2 and 17.8, 22.2, and 26.7°C ambient temperatures.

TABLE 23. SERUM CHEMISTRY AND THYROXINE DATA OF RATS EXPOSED TO VARIOUS LEVELS OF MICROWAVE RADIATION AT THREE ENVIRONMENTAL TEMPERATURES

Parameter	Exposure (mW/cm^2)	17.8°C		22.2°C		26.7°C	
		Mean	SE	Mean	SE	Mean	SE
Glucose	0	120.2	2.89	109.9	1.51	121.1	2.62
	5	122.5	2.15	116.5	1.78	118.9	3.19
	7.5	-	-	-	-	116.1	2.65
	10	120.8	2.55	108.8	2.98	-	-
	15	120.4	2.09	111.6	2.03	-	-
BUN	0	21.8	0.53	19.6	0.45	18.5	0.03
	5	18.4	0.34	17.7	0.47	17.5	0.60
	7.5	-	-	-	-	15.9	0.36
	10	17.1	0.46	17.5	1.03	-	-
	15	16.3	0.47	15.5	0.27	-	-
Creatinine	0	0.5	0.03	0.58	0.01	0.63	0.03
	5	0.5	0.02	0.57	0.02	0.60	0.02
	7.5	-	-	-	-	0.56	0.02
	10	0.5	0.03	0.55	0.02	-	-
	15	0.5	0.02	0.58	0.03	-	-
Na+	0	143.1	0.57	140.7	0.76	144.5	0.60
	5	141.9	0.60	141.1	0.95	143.4	0.45
	7.5	-	-	-	-	144.3	0.53
	10	142.5	0.84	141.6	0.67	-	-
	15	142.4	0.42	140.9	0.57	-	-
K+	0	6.01	0.27	5.54	0.19	6.07	0.12
	5	5.84	0.22	5.73	0.19	6.48	0.25
	7.5	-	-	-	-	6.04	0.11
	10	5.73	0.28	5.59	0.16	-	-
	15	5.62	0.20	5.37	0.21	-	-
Cl-	0	102.8	0.44	103.1	0.60	104.3	0.37
	5	102.9	0.23	103.2	0.51	104.6	0.45
	7.5	-	-	-	-	104.8	0.47
	10	103.6	0.26	104.4	0.37	-	-
	15	104.9	0.53	104.0	0.92	-	-

TABLE 23 (continued)

Parameter	Exposure (mW/cm^2)	17.8°C Mean	17.8°C SE	22.2°C Mean	22.2°C SE	26.7°C Mean	26.7°C SE
CO_2	0	30.0	0.63	29.1	0.60	26.9	0.57
	5	29.8	0.42	29.0	0.58	26.5	0.58
	7.5	-	-	-	-	27.3	0.84
	10	28.7	0.57	28.9	0.69	-	-
	15	27.8	0.47	28.9	0.52	-	-
Uric Acid	0	1.24	0.09	1.57	0.14	1.22	0.10
	5	1.30	0.05	1.54	0.14	1.33	0.08
	7.5	-	-	-	-	1.19	0.06
	10	1.25	0.08	1.59	0.11	-	-
	15	1.34	0.07	1.58	0.13	-	-
Ion Gap	0	10.3	0.88	8.50	0.88	13.3	0.30
	5	9.20	0.51	8.90	0.94	12.3	0.58
	7.5	-	-	-	-	12.3	0.22
	10	10.2	0.71	8.30	0.47	-	-
	15	9.78	0.85	8.00	0.99	-	-
Ionized Calcium	0	4.89	0.05	4.87	0.05	4.72	0.08
	5	4.93	0.04	4.87	0.03	4.34	0.09
	7.5	-	-	-	-	4.51	0.06
	10	4.87	0.04	4.80	0.03	-	-
	15	4.79	0.03	4.76	0.03	-	-
Calcium	0	10.1	0.03	10.1	0.02	9.80	0.04
	5	10.2	0.07	10.1	0.02	9.18	0.07
	7.5	-	-	-	-	9.53	0.11
	10	10.2	0.05	10.1	0.04	-	-
	15	10.0	0.06	9.90	0.05	-	-
P	0	6.86	0.21	6.27	0.17	6.00	0.37
	5	6.91	0.20	6.08	0.13	5.89	0.14
	7.5	-	-	-	-	5.86	0.11
	10	6.58	0.20	6.08	0.12	-	-
	15	6.63	0.14	6.10	0.11	-	-
Alka. Phos.	0	287.7	27.4	210.1	13.9	239.3	16.7
	5	218.0	12.0	206.4	9.58	183.7	13.9
	7.5	-	-	-	-	233.4	13.2
	10	206.6	15.8	196.3	10.3	-	-
	15	186.7	8.3	178.1	9.58	-	-
LDH	0	647.2	78.4	746.3	95.1	818.9	65.6
	5	608.8	70.0	568.2	31.6	771.2	51.6
	7.5	-	-	-	-	890.1	32.0
	10	618.5	82.8	727.0	78.8	-	-
	15	638.4	52.2	692.3	64.8	-	-
SGOT	0	'06.6	8.1	154.0	22.9	104.7	3.3
	5	100.4	4.8	105.8	4.0	121.9	15.0
	7.5	-	-	-	-	120.5	7.3
	10	108.5	9.2	119.3	8.6	-	-
	15	113.4	3.5	148.0	18.4	-	-

Parameter	Exposure	17.8°C		22.2°C		26.7°C	
	(mW/cm^2)	Mean	SE	Mean	SE	Mean	SE
SGPT	0	41.1	2.5	70.0	18.8	41.1	3.6
	5	34.6	2.1	40.1	1.2	52.5	14.8
	7.5	-	-	-	-	44.1	4.4
	10	36.8	2.8	45.2	4.7	-	-
	15	38.0	1.1	43.3	4.8	-	-
Cholesterol	0	102.1	4.8	107.1	4.8	93.7	3.4
	5	99.9	3.0	106.6	3.2	77.1	5.4
	7.5	-	-	-	-	79.0	5.2
	10	94.6	3.8	94.4	3.2	-	-
	15	90.0	4.4	91.6	5.6	-	-
Triglyceride	0	106.6	12.0	103.4	6.1	141.7	12.1
	5	92.8	9.8	103.8	5.9	127.7	8.2
	7.5	-	-	-	-	116.3	7.0
	10	99.8	6.0	98.3	4.9	-	-
	15	119.8	8.2	108.6	7.2	-	-
Total Protein	0	5.9	0.05	6.01	0.08	6.03	0.05
	5	5.9	0.04	6.04	0.06	6.16	0.09
	7.5	-	-	-	-	6.19	0.06
	10	6.0	0.07	6.20	0.07	-	-
	15	6.0	0.09	6.60	0.08	-	-
Albumin	0	3.26	0.02	3.37	0.04	3.33	0.03
	5	3.29	0.02	3.39	0.04	3.44	0.06
	7.5	-	-	-	-	3.43	0.05
	10	3.34	0.02	3.42	0.03	-	-
	15	3.31	0.06	3.36	0.04	-	-
Globulin	0	2.70	0.04	2.64	0.04	2.70	0.07
	5	2.67	0.03	2.65	0.04	2.72	0.06
	7.5	-	-	-	-	2.76	0.02
	10	2.73	0.05	2.78	0.05	-	-
	15	2.74	0.06	2.70	0.05	-	-
A/G	0	1.22	0.03	1.30	0.02	1.25	0.04
	5	1.24	0.02	1.29	0.03	1.27	0.03
	7.5	-	-	-	-	1.25	0.02
	10	1.22	0.02	1.25	0.02	-	-
	15	1.20	0.03	1.25	0.02	-	-
BUN/Cre	0	42.0	2.18	33.9	0.72	29.8	1.02
	5	36.7	1.58	31.3	0.32	29.2	0.31
	7.5	-	-	-	-	28.5	1.12
	10	34.9	1.77	31.9	1.61	-	-
	15	29.6	1.15	27.2	1.22	-	-
T4	0	5.01	0.20	4.46	0.35	3.96	0.27
	5	5.13	0.14	4.32	0.20	3.42	0.38
	7.5	-	-	-	-	2.96	0.28
	10	4.89	0.18	3.73	0.24	-	-
	15	4.39	0.28	3.64	0.42	-	-

TABLE 24. PROTEIN ELECTROPHORESIS DATA OF RATS EXPOSED TO VARIOUS LEVELS OF MICROWAVE RADIATION AT THREE ENVIRONMENTAL TEMPERATURES

Parameter	Exposure	17.8°C		22.2°C		26.7°C	
	(mW/cm^2)	Mean	SE	Mean	SE	Mean	SE
Albumin	0	54.1	1.20	51.6	1.51	55.0	0.89
	5	55.4	1.09	50.7	0.92	55.5	0.86
	7.5	-	-	-	-	55.8	3.11
	10	54.6	1.66	51.5	0.48	-	-
	15	55.3	1.39	53.1	1.29	-	-
Alpha 1	0	19.2	1.03	19.8	1.21	18.7	0.86
	5	18.3	0.81	21.0	0.84	19.5	0.86
	7.5	-	-	-	-	17.6	0.89
	10	20.0	1.46	19.7	0.77	-	-
	15	18.8	1.05	19.0	0.80	-	-
Alpha 2	0	6.70	0.45	7.60	0.34	6.20	0.39
	5	6.70	0.33	7.70	0.26	6.20	0.20
	7.5	-	-	-	-	5.63	0.23
	10	6.50	0.40	7.70	0.26	-	-
	15	5.89	0.19	7.50	0.22	-	-
Beta	0	15.6	0.43	16.1	0.48	15.4	0.27
	5	13.9	1.01	15.6	0.34	14.3	0.40
	7.5	-	-	-	-	15.8	0.37
	10	14.7	0.34	15.5	0.31	-	-
	15	15.3	0.41	15.1	0.41	-	-
Gamma	0	4.50	0.27	4.90	0.43	4.60	0.34
	5	4.60	0.22	4.90	0.28	4.50	0.27
	7.5	-	-	-	-	4.75	0.28
	10	4.20	0.42	5.60	0.37	-	-
	15	4.67	0.48	6.20	0.42	-	-
A/G	0	1.20	0.07	1.09	0.06	1.24	0.04
	5	1.24	0.07	1.04	0.03	1.25	0.04
	7.5	-	-	-	-	1.43	0.09
	10	1.24	0.08	1.07	0.03	-	-
	15	1.27	0.08	1.11	0.03	-	-

TABLE 25. HEMATOLOGY DATA OF RATS EXPOSED TO VARIOUS LEVELS OF MICROWAVE RADIATION AT THREE ENVIRONMENTAL TEMPERATURES

Parameter	Exposure	17.8°C		22.2°C		26.7°C	
	(mW/cm^2)	Mean	SE	Mean	SE	Mean	SE
WBC	0	5.88	0.53	5.38	0.40	10.5	4.52
	5	6.49	0.79	5.16	0.29	6.82	0.95
	7.5	-	-	-	-	5.75	0.29
	10	5.75	0.52	5.36	0.54	-	-
	15	4.51	0.38	5.28	0.40	-	-
RBC	0	7.70	0.11	7.51	0.04	7.50	0.28
	5	7.73	0.07	7.60	0.05	7.79	0.10
	7.5	-	-	-	-	7.67	0.42
	10	7.68	0.12	7.65	0.08	-	-
	15	7.47	0.12	7.35	0.20	-	-
HGB	0	14.9	0.24	14.6	0.11	13.7	0.68
	5	14.9	0.14	14.9	0.11	14.6	0.15
	7.5	-	-	-	-	14.7	0.18
	10	15.0	0.22	15.1	0.13	-	-
	15	14.9	0.12	14.9	0.11	-	-
HCT	0	42.0	0.89	40.8	0.43	38.4	2.70
	5	41.9	0.48	41.4	0.51	42.5	0.63
	7.5	-	-	-	-	41.9	0.74
	10	42.3	0.74	42.0	0.43	-	-
	15	42.4	0.76	42.0	0.45	-	-
MCV	0	54.5	0.76	54.3	0.43	51.7	1.56
	5	54.1	0.48	54.3	0.50	54.5	0.85
	7.5	-	-	-	-	54.4	1.35
	10	55.1	0.39	54.8	0.31	-	-
	15	56.7	0.34	55.7	0.64	-	-
MCH	0	19.4	0.26	19.5	0.15	18.5	0.27
	5	19.3	0.13	19.6	0.11	18.8	0.25
	7.5	-	-	-	-	19.2	0.33
	10	19.5	0.25	19.7	0.13	-	-
	15	19.9	0.22	19.8	0.12	-	-
MCHC	0	35.6	0.33	35.8	0.34	35.0	0.33
	5	35.6	0.40	36.0	0.22	34.5	0.28
	7.5	-	-	-	-	35.0	0.30
	10	35.5	0.53	35.9	0.20	-	-
	15	35.2	0.41	35.6	0.28	-	-
Lymphocyte	0	4.57	0.41	3.62	0.22	4.61	0.39
	5	5.49	0.69	3.85	0.28	4.85	0.66
	7.5	-	-	-	-	4.64	0.29
	10	4.44	0.62	3.42	0.57	-	-
	15	3.67	0.32	3.92	0.34	-	-
Neutrophil	0	1.12	0.23	1.62	0.26	1.12	0.20
	5	0.86	0.17	1.15	0.19	1.79	0.43
	7.5	-	-	-	-	0.89	0.13
	10	1.14	0.18	1.71	0.36	-	-
	15	0.71	0.11	1.15	0.17	-	-

TABLE 25 (continued)

Parameter	Exposure (mW/cm^2)	17.8°C Mean	17.8°C SE	22.2°C Mean	22.2°C SE	26.7°C Mean	26.7°C SE
Mono	0	118.4	34.0	77.6	39.6	130.1	29.0
	5	60.7	23.2	77.7	25.0	132.6	53.9
	7.5	-	-	-	-	124.3	33.5
	10	57.2	22.2	113.7	43.2	-	-
	15	114.6	34.1	122.1	59.5	-	-
Eosin	0	40.5	15.6	33.0	12.0	57.4	18.7
	5	68.1	26.1	86.7	38.4	49.2	20.0
	7.5	-	-	-	-	87.5	28.1
	10	95.2	31.2	39.4	18.6	-	-
	15	20.1	14.2	22.4	17.1	-	-

Statistical analysis using analysis of variance was done on each parameter according to the same arrangement as described before: 1) 17.8 and 22.2°C and 0, 5, 10, and 15 mW/cm^2; 2) 17.8, 22.2, and 26.7°C and 0 and 5 mW/cm^2; 3) 26.7°C and 0, 5, and 7.5 mW/cm^2.

Table 26 lists the statistical results of these analyses. Most effects were due to temperature change. Consistent temperature effects observed in sets 1 and 2 are on glucose, BUN, creatinine, sodium, uric acid, ion gap, phosphorus, alkaline phosphatase, albumin, BUN-to-creatine ratio, T_4, alpha 2, A/G ratio, and lymphocyte. Three parameters consistently show radiation effects in all three sets: BUN, ionized calcium, alkaline phosphatase. None of the protein electrophoresis and hematology parameters were affected by the up-to-lethal level of microwave radiation and high environmental temperature.

TABLE 26. STATISTICAL RESULTS OF ANIMAL HEALTH PROFILE

Parameter	Set 1		Set 2		Set 3	
	T	R	T	R	TR	R
Glucose	<0.0001		0.0030			
BUN	0.0366	<0.0001	0.0001	<0.0001	0.0394	0.0024
Creatinine	0.0037		0.0004			
Na+	0.0060		0.0002			
K+			0.0138			
Cl-				0.0146		
CO2			<0.0001			
Uric Acid	0.0004		0.0122			
Ion Gap	0.0132		<0.0001			
Ionized Ca		0.0108	<0.0001	0.0284	0.0019	0.0111
Calcium		0.0187	<0.0001		0.0125	
P	<0.0001		<0.0001			
Alka. Phos.	0.0115	0.0003	0.0156	0.0025		0.0276
LDH			0.0405			
SGOT	0.0054	0.0684			0.0287	
SGPT	0.0234					
Cholesterol		0.0032	<0.0001			0.0394
Triglycerides			0.0007			
Total Protein						
Albumin	0.0016		0.0056			
Globulin						
A/G	0.0020					
BUN/Cre	<0.0001	<0.0001	<0.0001	0.0128		
T4	0.0001	0.0235	<0.0001			
Albumin	0.0007		0.0008			
Alpha 1						
Alpha 2	<0.0001		0.0003			
Beta				0.0168		
Gamma	0.0010					
A/G	0.0002		0.0023			
WBC						
RBC						
HGB			0.0431			
HCT						
MCV		0.0009				
MCH		0.0472				
MCHC			0.0173			
Lymphocyte	0.0065		0.0412			
Neutrophil	0.0055					
Mono						
Eosin						

T: Temperature effect
R: Radiation effect
TR: Temperature - radiation effect

The means and standard errors of the corticosterone level in rats exposed to various incident power densities and environmental temperatures are shown in Figure 25. Analysis of variance of the data at each temperature showed significant difference only at 17.8°C ($f = 3.43$, $df = 3,35$, $p < 0.05$). For the sham and 5-mW/cm^2 groups, two-way analysis of variance detected no effect of temperature or exposure.

At low temperature (17.8°C), the corticosterone level was low (2.1 µg/100 ml) but increased while exposed to microwaves, especially at 15 mW/cm^2. The difference was statistically significant. At 22.2°C, however, the level decreased when compared to control, although not statistically significant. Animals died during the 15-mW/cm^2 exposure at medium and high temperatures; obviously this was highly stressful. Then why did the corticosterone level not show this effect since it is related to stress? Possibly the observed effect was due to adrenal exhaustion when exposed to chronic stress (Selye, 1950). This is often seen in aging rats, when the pituitary-adrenal-system functioning remains intact but the reserve capacity to respond to stress is diminished (Hess and Riegle, 1970).

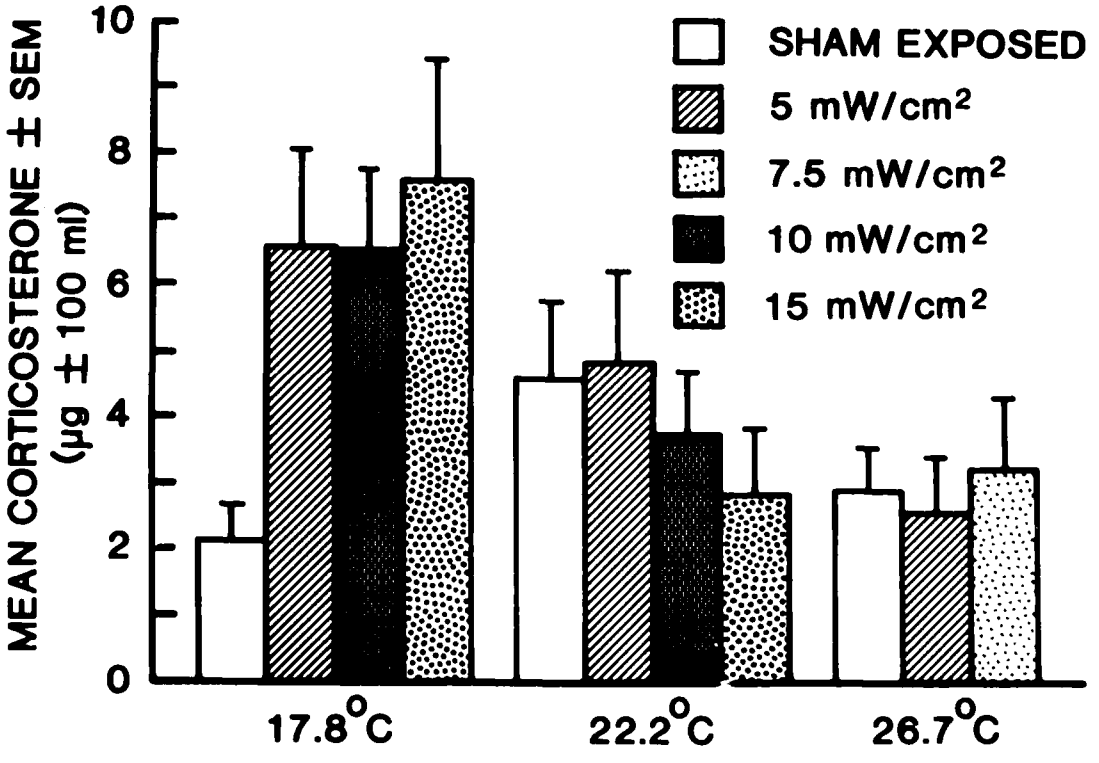

Figure 25. Corticosterone levels in rats exposed to various levels of microwave radiation under three environmental temperatures.

Oxygen Consumption and Carbon Dioxide Production

Table 27 lists the O_2 consumption and CO_2 production of the rats exposed to 5, 7.5, 10, or 15 mW/cm^2 at 17.8, 22.2, or 26.7°C. No data for sham-exposed rats are shown. When the sham-exposed rat was in the metabolism cage, the empty cage provided no data that could be used for the correction of the drift in equipment. However, when the sham and exposed animals were measured simultaneously, the differences between them were obtained, as shown in Table 28. An example of daily variations of the data is shown in Figure 26. The data show a consistent decrease of both O_2 and CO_2 production for all exposures.

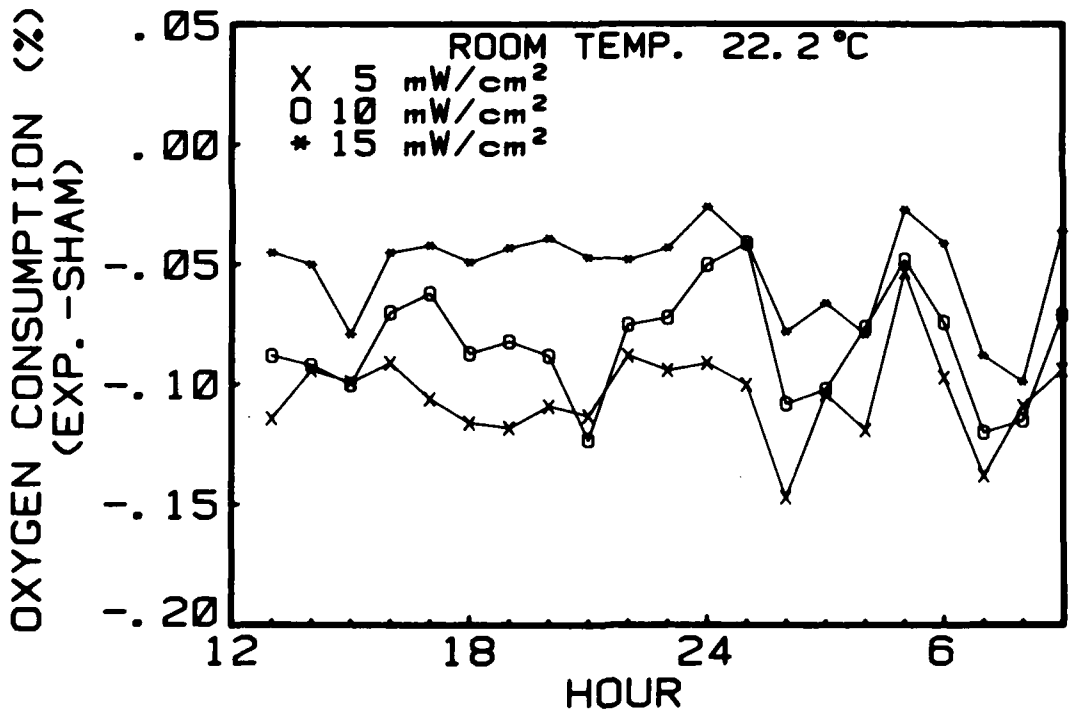

Figure 26. Example of daily oxygen-consumption variation (averaged over 6 wk) at 22.2°C.

TABLE 27. OXYGEN CONSUMPTION (%) AND CARBON DIOXIDE PRODUCTION (%) OF RATS EXPOSED TO MICROWAVE RADIATION UNDER THREE ENVIRONMENTAL TEMPERATURES

Exposure (mW/cm^2)	Time	17.8°C		22.2°C		26.7°C	
		Mean	SD	Mean	SD	Mean	SD
				O_2 Consumption			
5	e	0.325	0.022	0.348	0.011	0.369	0.047
	l	0.307	0.021	0.388	0.022	0.323	0.083
7.5	e					0.382	0.043
	l					0.305	0.128
10	e	0.308	0.055	0.350	0.017		
	l	0.320	0.016	0.397	0.023		
15	e	0.278	0.053	0.367	0.072		
	l	0.277	0.031	0.427	0.039		
				CO_2 Production			
5	e	0.205	0.065	0.241	0.026	0.160	0.013
	l	0.223	0.013	0.247	0.022	0.153	0.022
7.5	e					0.164	0.015
	l					0.130	0.057
10	e	0.172	0.056	0.213	0.023		
	l	0.192	0.007	0.224	0.028		
15	e	0.173	0.055	0.217	0.032		
	l	0.196	0.015	0.247	0.022		

e and l = early and late recordings for 6-wk experiment

TABLE 28. CHANGE OF OXYGEN CONSUMPTION (%) AND CARBON DIOXIDE PRODUCTION (%) OF RATS EXPOSED TO MICROWAVE RADIATION, RELATIVE TO SHAM-EXPOSED RATS, UNDER THREE ENVIRONMENTAL TEMPERATURES

Exposure (mW/cm^2)	Time	17.8°C		22.2°C		26.7°C	
		Mean	SD	Mean	SD	Mean	SD
				O_2 Consumption			
5	e	-0.068	0.080	-0.122	0.032	-0.053	0.053
	l	-0.068	0.020	-0.101	0.005	-0.037	0.013
7.5	e					-0.075	0.040
	l					-0.030	0.022
10	e	-0.093	0.031	-0.101	0.011		
	l	-0.115	0.022	-0.042	0.011		
15	e	-0.105	0.079	-0.090	0.004		
	l	-0.122	0.014	-0.045	0.010		
				CO_2 Production			
5	e	-0.030	0.032	-0.091	0.013	-0.043	0.019
	l	-0.050	0.030	-0.065	0.001	-0.047	0.016
7.5	e					-0.054	0.020
	l					-0.053	0.001
10	e	-0.072	0.043	-0.113	0.001		
		-0.099	0.011	-0.083	0.017		
15	e	-0.059	0.037	-0.108	0.005		
	l	-0.075	0.014	-0.078	0.019		

e and l = early and late recordings for 6-wk experiment

AD-A169 064 EFFECTS OF LONG-TERM RADIOFREQUENCY RADIATION ON IMMUNOLOGICAL COMPETENCE..(U) UNIVERSITY HOSPITAL SEATTLE WA BIOELECTROMAGNETICS RESEARCH L.. 2/2

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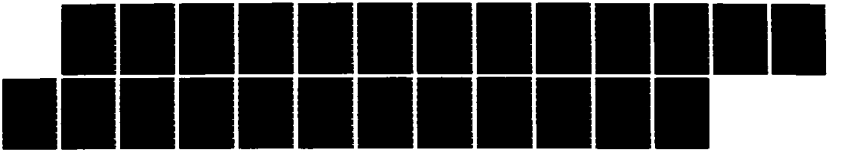

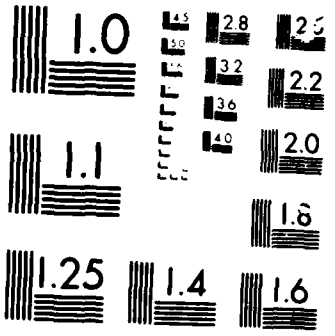
1.0
2.8
3.2
3.6
4.0
2.2
2.0
1.8
1.1
1.25
1.4
1.6

Statistical analysis of the O_2 data was done by sets as before, except there was no sham-exposed group here. In rats exposed to 5, 10, or 15 mW/cm^2 at 17.8 and 22.2°C, the effects of temperature ($f = 98.9$, $df = 1,36$, $p < 0.0001$); temperature-radiation ($f = 7.44$, $df = 2,36$, $p = 0.002$); time ($f = 7.96$, $df = 1,36$, $p = 0.0077$); and time-temperature ($f = 9.54$, $df = 1,36$, $p = 0.039$) were significant. Summaries of least significant differences are shown in Table 29. Differences in O_2 consumption were not significant between underscored conditions; differences not underlined were statistically significant at $p < 0.01$. When analyzed separately for 17.8 and 22.2°C, the effect of radiation was significant for 17.8 ($f = 5.22$, $df = 2,18$, $p = 0.0163$) but not for 22.2°C. At 22.2°C there was a significant effect of time ($f = 17.56$, $df = 1,18$, $p = 0.0006$).

TABLE 29. COMPARISON OF OXYGEN CONSUMPTION IN RATS EXPOSED TO 5, 10, OR 15 mW/cm^2 AT 17.8°C (L) OR 22.2°C (M)

a) Averaged across time

L - 15 L - 10 L - 5 M - 5 M - 10 M - 15

b) Averaged across radiation

l - L e - L e - M l - M

Magnitudes increase from left to right
e,l = early and late recordings

At 5-mW/cm^2 exposure, the effect of temperature alone was not significant, but it was significant for time-temperature interaction ($f = 9.83$, $df = 2,18$, $p = 0.0013$). The groups are (l-L, l-H, e-L, e-M, e-H) and (e-L, e-M, e-H, l-M). Radiation had no effect on O_2 consumption at 26.7°C (H), but early consumption was significantly higher than late consumption ($f = 6.23$, $df = 1,11$, $p = 0.0297$).

Carbon dioxide production was analyzed in the same way. In rats exposed to three power densities at 17.8 and 22.2°C, effects of temperature ($f = 23.65$, $df = 1,36$, $p < 0.0001$); radiation ($f = 4.71$, $df = 2.36$, $p = 0.0151$); and time ($f = 5.91$, $df = 1,36$, $p = 0.0202$) were significant. When compressed over time, the data can be grouped into "17.8°C: 10, 15, 5 mW/cm^2" and "17.8°C: 5 mW/cm^2; 22.2°C: 10, 15, 5 mW/cm^2." When analyzed separately for 17.8 and 22.2°C, the effect of radiation was not significant for either temperature. The time effect at 22.2°C showed higher CO_2 production late in the exposure rather than early. The effect of temperature at 5-mW/cm^2 exposure was highly significant ($f = 25.77$, $df = 2,18$ $p < 0.0001$). Data can be grouped into "26.7°C" and "17.8°C, 22.2°C." At 26.7°C, radiation had no effect on CO_2 production.

Immunological Competence Evaluation

Statistical Methods. Rats available for study at the end of each of the three 6-wk temperature experiments were killed and assayed according to the following plan:

Temp	Kill day	5 mW/cm^2		10 mW/cm^2		15 mW/cm^2		Shams Pooled
		SH	EXP	SH	EXP	SH	EXP	
17.8°C	1	1	3	1	3	1	2	3
17.8°C	2	1	3	1	3	1	3	3
17.8°C	3	2	4	1	4	1	4	4
TOTAL		4	10	3	10	3	9	10
22.2°C	4	1	3	1	3	1	3	3
22.2°C	5	2	4	1	4	1	4	4
22.2°C	6	1	3	1	3	1	3	3
TOTAL		4	10	3	10	3	10	10
						7.5 mW/cm^2		
26.7°C	7	-	5	3	-	2	4	5
26.7°C	8	4	5	-	-	1	4	5
TOTAL		4	10	3	-	3	8	10

Units of variation were all based on one observation per animal. For the flow cytometric variables, each observation consisted of a single measurement from one 20,000-cell sample analyzed. For the in vitro function tests of lymphocyte activation, each observation was the average of three determinations for an individual rat. For the CFU-C assay, each observation was an average of two replicate samples.

Data for each variable were considered in two analysis-of-variance (ANOVA) contexts. Sham and exposed rats (0 and 5, 10, and 15 mW/cm^2 respectively) observed at 17.8°C and 22.2°C were included in a general mixed-model design that was analyzed via maximum-likelihood estimation (MLE) under the BMDP3V statistical computer program (BMDP Statistical Software, 1983). Radiation dosage and temperature served as completely crossed fixed factors with four and two levels respectively. Day of sacrifice served as a random factor with six levels (days); three levels nested in each temperature level and completely crossed with the four radiation levels. Control (sham) animals were pooled across alcoves with sacrifice-day membership preserved to achieve a reasonably balanced design. However, complete or proportional balance was still unobtainable, thus necessitating the MLE approach to the mixed model. The model saturated in the fixed effects (RFR, temperature, RFR-temperature interaction) and all possible subset models controlling for day of sacrifice were evaluated via change in deviance under the Chi-square likelihood ratio test and subsequent consideration of MLE/standard-error ratio for individual effects. In a similar manner significance of the random effect associated with day of sacrifice was evaluated against the fixed-effects saturated model. In order to identify outlier-dependent effects, all models were evaluated with and without extreme outliers.

Sham and exposed (5 and 7.5 mW/cm^2) rats observed at 26.7°C were included in a proportionally balanced mixed-model design that was analyzed via standard ANOVA methodology. Radiation dosage served as a fixed factor with three levels. Day of sacrifice served as a random factor with two levels which were completely crossed with the three radiation levels. Sham animals were again pooled across alcoves with sacrifice-day membership preserved. In this instance proportional balance in cell size was achieved, insuring orthogonality of sums of squares. This allowed evaluation of overall F tests for interaction and main effects of the fixed and random factors. Data sets with extreme outliers were reanalyzed in a Winsorized fashion in order to identify outlier-dependent effects.

Results

The statistical model used in this study indicated that statistically significant temperature and radiation effects were associated with many of the variables considered. Close examination of respective MLEs and their standard errors showed that only some of the effects were of biological significance; the raw data is presented. Table 30 shows the effects of radiation and temperature on cellularity of the spleen and thymus. With the exception of the spleen at 0 mW and 17.8°C, at any one exposure level, cellularity with these organs tended to decrease as temperature increased. However, increasing radiation dose from 0 to 5 and then 5 to 10 mW/cm^2 tended to increase cellularity. At the 15-mW/cm^2 dosage, the number of cells decreased to below numbers for the unexposed group. For the spleen the radiation effect was significant ($p < 0.05$), and for the thymus the radiation and temperature effects were significant ($p < 0.05$ each).

The following is a list of statistical-effects ($p < 0.05$) abbreviations used in the tables.

		Outlier dependent
Radiation	R	r
Temperature	T	t
Day	D	d
Interactions	RT, RD	rt, rd
No effect	-	
Not calculated	X	

TABLE 30. CELLULARITY OF THE SPLEEN AND THYMUS IN ANIMALS UNEXPOSED AND EXPOSED TO RADIOFREQUENCY RADIATION

Exposure level (mW/cm^2)	Cells counts per organ x 10^6 [mean(SD)]			
	Spleen[a]		Thymus[b]	
	17.8°C	22.2°C	17.8°C	22.2°C
0	211(44)	226(62)	232(126)	145(64)
5	225(42)	198(67)	296(72)	180(75)
10	213(69)	204(64)	250(74)	150(95)
15	158(60)	131(25)	181(50)	132(66)

a: R
b: R, T

The response of hematopoietic progenitor cells to radiation and temperature conditions is depicted in Table 31. Radiation and temperature had significant effects on the numbers of macrophage and total colonies generated. For example, the number of macrophage colonies rose from 78 and 38 at 0 dosage to 105 and 46 at 10 mW/cm^2, respectively, at 17.8 and 22.2°C; at 15 mW/cm^2 the responses were equal to or lower than control values. This same pattern of responsiveness was observed for the granulocyte, mixed, and total-colony responses. The temperature effects within a given exposure group reveal that macrophage, granulocyte and total-colony responses each decreased approximately 50%; the shams also responded in this manner. Statistical analysis revealed an interactive effect between temperature and radiation in the response of mixed-colony progenitors. The exact biological significance of this is unclear.

TABLE 31. RESPONSE OF HEMATOPOIETIC PROGENITOR CELLS TO VARYING TEMPERATURES AND RFR DOSES

Exposure level (mW/cm^2)	Number of CFU per 10^5 Marrow Cells Plated(SD)			
	17.8°C	22.2°C	17.8°C	22.2°C
	Macrophage		Granulocyte	
0	78(19)[a]	38(7)	19(8)[b]	6(6)
5	93(16)	43(18)	30(13)	9(6)
10	105(25)	46(9)	24(7)	9(2)
15	77(20)	32(12)	30(14)	7(3)
	Mixed		Total	
0	14(7)[c]	18(8)	111(20)[d]	61(12)
5	20(8)	16(6)	143(35)	68(27)
10	14(3)	31(7)	143(25)	86(13)
15	19(5)	20(10)	125(37)	58(17)

a = rt, R, T, D
b = r, T, d
c = RT, R, T, D
d = R, T, D.

The functional activity of T- and B-cell lymphocyte populations from the spleen and thymus was examined by stimulation in vitro with various mitogens such as LPS, Con A, PWM, PHA, and PPD. Although statistically significant effects (RT, R, and T) were observed in many of the mitogen variables, close examination of respective MLEs and their standard errors revealed no biologically meaningful differences in any of the "statistical cells" analyzed. Table 32 summarizes the statistical effects that were found. Immunofluorescence flow cytometric analysis of the populations of cells in the bone marrow, spleen, and thymus gland, using antisera-defining cell-surface differentiation antigens present on B cells (s-Ig) and T cells (Thy 1.1), is summarized in Table 33.

TABLE 32. SUMMARY OF STATISTICAL EFFECTS FOUND BY VARYING THE TEMPERATURE AND DOSE OF RFR ON THE IN VITRO MITOGEN RESPONSIVENESS OF SPLENOCYTES AND THYMOCYTES

Spleen	17.8-22.2°C	26.7°C	Thymus	17.8-22.2°C	26.7°C
			Observed scale		
LPS	-	RD	Con A	D	R
Con A	RT, D	-	PWM	D	r
PWM	T	D	PHA	T, d	rd, R
PHA	R	RD			
PPD	T	D			
			Log Scale		
LPS	-	RD, r	Con A	D	D
Con A	RT, D	RD, R	PWM	T, D	R
PWM	T	RD, R	PHA	T, D	RD
PHA	R	RD			
PPD	t	D			

TABLE 33. SUMMARY OF STATISTICAL EFFECTS FOR THE FLOW CYTOMETRIC DATA COMPARING MARROW, THYMUS, AND SPLENOCYTE B- AND T-CELL POPULATIONS UNDER VARYING CONDITIONS OF TEMPERATURE AND RFR

		Significant Effects Observed					
		Bone Marrow		Spleen		Thymus	
Stain	Population analyzed	17.8-22.2°C	26.7°C	17.8-22.2°C	26.7°C	17.8-22.2°C	26.7°C
B cell	Viable cells						
	Peak intensity	D	D	-	D	D	X
	Mean intensity	D	D	D	D	D	RD
	% lymphocytes	T, D	D	R, D	D	D	R, D
	Peak lymphocytes	r, T, D	D	D	D	D	X
	Mean lymphocytes	R, D	D	D	D	D	D
	% viable cell with s-Ig	T, d	R, D	D	-	RT, D	R
	% lymphocyte with s-Ig	R, D	RD,	D	D	RT, D	-
T cell	Viable cells						
	Peak intensity	T	D	T, D		d	R
	Mean intensity	T, D	D	R, T, D	-	D	R, D
	% lymphocytes	T, D	-	T, D	-	rt, r T, D	D
	Peak lymphocytes	R, D	D	D	D	d	D
	Mean lymphocytes	T, D	R, D	RT, R, T, D	D	D	R, D
	% viable cell with Thy 1.1	D	R, D	T, D	D	r, D	-
	% lymphocyte with Thy 1.1	D	R, D	D	D	rt, r, T, d	-

Only a summary form of the statistical analysis is presented since few biologically meaningful differences were observed. Those differences deemed important are shown in Tables 34 and 35. In Table 34 a pronounced temperature effect can be observed when the percent of B cells expressed as a fraction of all viable marrow cells is considered as a function of temperature and radiation exposure levels. General trends included an increasing fraction of B cells at 22°C and a substantial decrease at 26.7°C. A radiation effect was noted only at 26.7°C. In Table 35 a clear-cut radiation effect is evident when the fraction of B cells of all viable cells is examined as a function of radiation dose. From 0 to 7.5 mW/cm^2, the fraction of B cells increased from 2% to 5% of all cells.

TABLE 34. FRACTION OF BONE MARROW CELLS DETECTED BY FLOW CYTOMETRIC TECHNIQUES USING ANTIBODIES SPECIFIC FOR B CELLS

Exposure level (mW/cm^2)	% B cells (SD)		
	17.8°C[a]	22.2°C[a]	26.7°C[b]
0	12(5)	13(2)	8(2)
5	10(2)	12(2)	10(3)
7.5	c	c	7(2)
10	10(2)	14(3)	c
15	11(1)	14(3)	c

a = T
b = R
c = not tested

TABLE 35. FRACTION OF THYMOCYTES DETECTED BY FLOW CYTOMETRIC TECHNIQUES USING ANTIBODIES SPECIFIC FOR B CELLS

Exposure Level (mW/cm^2/26.7°C)	% B cells(SD)
0	2(2)
5	3(3)
7.5	5(3)

R, p = 0.055

This study was designed to evaluate the immunologic status of rats exposed to three environmental temperatures (17.8, 22.2, and 26.7°C) and to 0, 5-, 10-, and 15-mW/cm^2 RFR over a 6-wk period. Alterations in the hematopoietic and immunologic networks have been reported in animals exposed to RFR at and below 10 mW/cm^2, but such effects could be attributable to thermal changes within the animals. The experiments we report here demonstrate alterations in the hematopoietic and immunologic systems of rats exposed to various temperatures and levels of RFR. Dramatic effects observed were as follows: (a) A pronounced radiation effect ($p < 0.05$) on the cellularity of the spleen where a 30% decrease in the numbers of recoverable viable cells occurred after 15-mW/cm^2 exposure; elevating the temperature from 17.8 to 22.2°C did not produce any additional changes. (b) A dramatic radiation and temperature effect for the cellularity of the thymus gland in which a biphasic response was noted. Exposure to 5 mW/cm^2 at both 17.8 and 22.2°C produced a 20 to 30% increase in numbers of recoverable viable thymocytes compared to the unexposed animals; however, the elevation in temperature alone produced nearly 30% reductions in cellularity. Stimulatory and inhibitory effects of RFR on the immune response have been reported previously although not under these experimental conditions. Bowhill (1981) and Smialowicz (1982a) suggest that immunological competence tends to change in rats exposed to microwave radiation at any SAR in excess of their basal metabolic rate. Bowhill has postulated a biphasic response of the immunological apparatus after RFR exposure. Our data is consistent with such a hypothesis. The basis for this response could best be explained by thermal stress and the ensuing process of thermoregulation.

The response of the hematopoietic progenitor cells of the marrow to these conditions was very different from that after 6 and 12 mo of exposure to low levels of RFR (480 μW/cm^2). The numbers of total colonies and macrophage, mixed, and granulocyte colonies from animals exposed at 17.8°C demonstrated a significant radiation effect ($p < 0.05$) at low RFR levels (5 and 10 mW/cm^2) where the number of colonies increased (20-70%)

but then decreased somewhat. The response at 22.2°C was decreased by 2- to 3-fold at every exposure level, with the exception of the mixed-colony response which appeared insensitive to the effects of elevated temperature. Comparing these progenitor cell responses to those of animals exposed to low levels of RFR for 6 and 12 mo reveals differences. Although colony types were reduced in numbers and were distributed differently, in this study a 10-fold increase in the RFR dose generally produced a 30-70% increase in colonies. This effect was temperature dependent but not observed consistently at 22.2°C. The biological basis for these findings is unclear and awaits further investigation.

Among the many variables examined by immunofluorescence cytometry, only two appeared to have significant biological implications. The first is a decrease in the percentage of B cells in the marrow, which was independent of radiation but highly dependent on temperature. With no RFR a temperature increase of 4.4°C produced a 30% decrease in marrow B cells. No such effect was seen in exposed animals. The frequency of B cells in the thymus gland is generally low (< 2.0%); the levels of thymic B cells in sham-exposed animals were consistent with this observation. Exposure to 7.5 mW/cm^2 of RFR, however, doubled the levels of thymic B cells. In the previous 6- and 12-mo study conducted at the University of Washington, the composition of the lymphoid cells within the marrow underwent only relatively minor alterations--RFR produced a marginally significant ($p = 0.05$) reduction in the fraction of marrow B cells. Our observation of increased levels of B cells within the thymus gland is in marked contrast to that of the previous study after 12-mo exposure. That microwave radiation produces alterations in the migration of lymphoid cells has been reported. Such an explanation might apply to our findings of increased B cells in the thymus. Several interpretations exist, but the one most plausible is that radiation increases B-cell migration to the thymus from the blood. Further experiments would be required to substantiate this notion.

The statistical approach used in this study requires some comment. The general mixed-model design for the experiments at 17.8°C and 22.2°C was analyzed via maximum-likelihood estimation. This method was necessary to

account for random-day effects against the fixed effects of RFR, temperature, and RFR-temperature interaction in an unbalanced design. Due to the small number of animals used in this study, the biological significance of variable effects with $p < 0.05$ was posited by examining the magnitude and variability of the MLEs for these effects. For many of these variables no body of literature exists that defines normal values and the expected variations within a normal rat population. Some variables measured are intrinsically precise and accurate; for example, the flow cytometric variables generally show small variations (< 10%) about means. By contrast, measurements of mitogen stimulation of cells in vitro usually show much greater variation (30-80%) about the means. The magnitude of responses considered to be biologically meaningful can be seen in the AIDS literature where absence or reduction of response by 80% are deemed biologically significant.

Gross Pathological and Histopathological Evaluation

Tables 36-38 list the lesions of the animals exposed to 0, 5, 7.5, 10, and 15 mW/cm^2 at 17.8, 22.2, and 26.7°C environment. Necropsy of the animals that died during the exposure showed pulmonary hemorrhage and edema apparently due to the heat stress. For all three temperatures, high lesions occurred in the lungs. Since each temperature study lasted only 6 wk, not many lesions were found. Statistical analysis would not be meaningful because of not only the sparse data but also the uneven sample size and exposure time due to early death during high-power exposure.

TABLE 36. HISTOPATHOLOGICAL RESULTS OF 6-WEEK EXPOSURE* AT 17.8°C

Lesions	Power Density (mW/cm^2) 0	5	10	15
Adrenal				
foci of cortical cellular alteration	1			
Aorta				
multifocal and mineralization				1
Epididymus				
sperm granuloma			2	
Kidney				
membranous glomerulonephritis				1
cystic collecting tubule	4	2	2	2
Liver				
acute hepatic congestion				1
Lung				
acute pulmonary congestion and edema				1
acute petechial agonal pulmonary hemorrhages				1
peribronchiolar lymphoreticular cell proliferation	6	7	7	7
Mandibular sg				
nonsuppurative periductal sialoadenitis	1	1	1	
Pancreas				
pancreatic ductal lithiasis			1	1
Stomach				
diffuse gastric hyperkeratosis			1	
gastric hyperkeratosis		1		1
Thymus				
multiple echymotic hemorrhages				1

*Ten animals in each group.

TABLE 37. HISTOPATHOLOGICAL RESULTS OF 6-WEEK EXPOSURE* AT 22.2°C

Lesions	Power Density (mW/cm^2) 0	5	10	15
Adrenal				
focal adrenal cortical ossification			1	
foci of cellular alteration	2			1
Brain				
pituitary cyst				1
Heart				
cardiomyopathy		1		
Kidney				
cystic tubules	2		1	1
Liver				
nonsuppurative hepatitis	1			
Lung				
peribronchiolar lymphoid cell proliferation	9	7	7	5
Pancreas				
cystic pancreatic ductal lithiasis				1
Pituitary				
cyst	1			
Salivary gland				
nonsuppurative periductal adenitis	1		1	

*Ten animals in each group.

TABLE 38. HISTOPATHOLOGICAL RESULTS OF 6-WEEK EXPOSURE* AT 26.7°C

Lesions	Power Density (mW/cm^2) 0	5	7.5	10
Adrenal				
foci of cellular alteration	1			
Heart				
focal cardiomyopathy	1	1		
focal cardiomyopathy				1
focal ectopic myocardial ossification			1	
Kidney				
hydronephrosis	1			
renal collecting tubules	1			
renal cortical cyst		1		
multiple cystic collecting tubules		1		
Liver				
peribronchiolar lymphoreticular cell proliferation			1	
Lung				
acute pulmonary congestion				1
peribronchiolar lymphoreticular cell proliferation	9	8	5	
Pancreas				
pancreatic ductal lithiasis	1			
Preputual gland				
cystic preputual gland hyperplasia	1			
Stomach				
gastric hyperkeratosis	1			
Urethra				
proteinaceous urethral calculi				1
Zymbals gland				
nonsuppurative periductal adenitis			1	

*Number of animals in each group: 0 (10)
5 (10)
7.5 (8)
10 (1)

CONCLUSIONS

Effects observed in the original long-term exposure study--the increased corticosterone at 6 wk, the increased B and T cells after 13 mo, and the effect on mitogen stimulation--were not replicated in this study. Instead, the only consistent effect that we observed in both 6- and 12-mo exposed rats was the increase in hematopoietic progenitor cells in their bone marrow. Confirmation and further studies of this effect are needed.

In this study when both the radiation and environmental temperature were raised to high levels, the effects seen were mainly on thermoregulation which is a complex function of power level and environmental temperature.

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